

III B.Sc VI SEMESTER BOTANY PAPER-VII
Plant physiology and Metabolism



**SREE SIDDAGANGA COLLEGE
OF ARTS, SCIENCE and COMMERCE
B.H. ROAD, TUMKUR
(AFFILIATED TO TUMKUR UNIVERSITY)**



**BOTANY PAPER-VII
III BSC VI SEMESTER
STUDY MATERIAL**

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Plant physiology and Metabolism

II BSc IV SEMESTER BOTANY CBCS QUESTION BANK

Unit 1 Plant-water relations (6 Hrs)

Importance of water, water potential and its components;

A brief account of absorption of water [active and passive] and Ascent of sap[transpiration pull theory]

Transpiration: Structure of stomata, Stomata mechanism (Steward and K- ion theory) Factors affecting transpiration; Anti-transpirants.

Unit2- Mineral nutrition (3 Hrs)

Essential elements, macro and micronutrients; Role and deficiency symptoms of Nitrogen, phosphorus, Potassium, Magnesium, Zinc, boron, and Molybdenum: Hydroponics

Unit 3- Photosynthesis (10 Hrs)

Photosynthetic apparatus, Photosynthetic Pigments (Chl a, b, xanthophylls, carotene); Photo system I and II, reaction centre, antenna molecules; Electron transport and mechanism of ATP synthesis; C3 , C4 and CAM pathways of carbon fixation.

Unit 4- Respiration (6 Hrs)

Structure of mitochondrion, Glycolysis, anaerobic respiration, TCA cycle; Oxidative Phosphorylation, Pentose Phosphate Pathway.

Unit 5: Enzymes (5 Hrs)

Structure, Nomenclature, Properties, classification; Mechanism of enzyme action and enzyme inhibition.

Unit 6: Nitrogen metabolism (4 Hrs)

Biological nitrogen fixation; Nitrate Metabolism, Synthesis of amino acids, Reductive and Transamination.

Unit 7 Plant growth regulators (4 Hrs)

Auxins, Gibberellins, Cytokinins, Ethylene, ABA and their role in agriculture and horticulture.

Unit 8: Plant response to light and temperature (4 Hrs)

Photoperiodism, Phytochromes, Florigen concept, Vernalization.

Unit 9: Dormancy : (1 Hour)

a brief account of seed dormancy

Unit 10: Plant movements: (2 Hrs)

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(Phototropism, geotropism, hydrotropism and seismonasty)

V Semester- Paper –VII

Plant Physiology and Metabolism

Unit 1: Plant-water relations

Importance of water, water potential and its components;

A brief account of absorption of water [active and passive] and Ascent of sap[transpiration pull theory]

Importance of water

water potential and its components

A Brief account of Absorption of Water by Roots

Water Absorption: Organ, Mechanism: - Absorption of water and nutrient is carried by the younger portions of a root, near its tip. The very tip is covered with a sheathing root cap which protects the delicate underlying tissues as the root pushes its way through the soil.

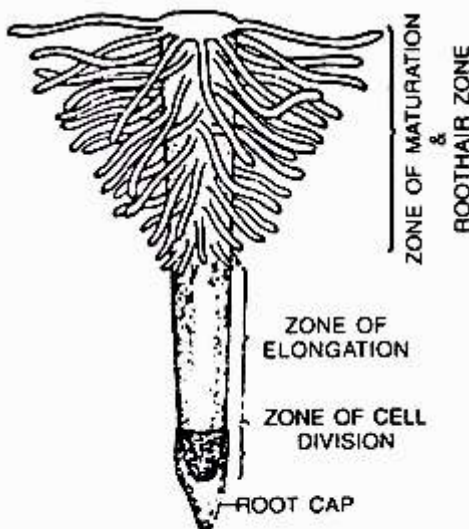


Fig. 2.2 Tip of a young root magnified to show various zones.

Besides root cap, three more zones can be recognized in the young root tip.

These zones are:

Zone of cell division or meristematic zone: The thin-walled cells of this region are alike and constantly divide, results in increases in length of root.

The zone of elongation: The cells formed in the meristematic region become longer in the region of elongation.

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The zone of maturation & Root Hair Zone: In this region Some cells take on structural features which enable them to conduct water. Others become specialized for the conduction of food, and still others for food storage.

Root hairs develop in the younger part of the maturation zone as finger-like extensions of the epiblema cells .

Introduction

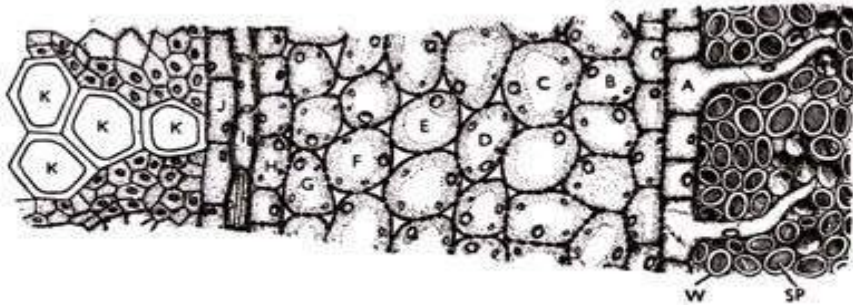


FIG. 671. Path of water from the soil through the root hair A, the layers of cortex B, C, etc., to the xylem vessels, K; W, films of water around soil particles, Sp.

If the root hair has a high osmotic pressure and is exposed to the surrounding soil water in the soil , the cells and the xylem vessel have lower pressures. water will pass from root hair to xylem by diffusion from the soil to the roots following the same osmotic relations. Thus, the root hair will take up water from the surrounding medium as water move by diffusion from regions of higher water potential to regions of lower water potential.

The force with which water will be drawn from the soil will depend entirely upon the difference between the osmotic pressure external and the osmotic pressure of the xylem vessels. The greater the difference, the greater will be the force with which water is drawn into the vessels through the cortical cells.

Generally the osmotic pressure of the cell sap of the xylem can scarcely reach a value as low as the osmotic pressure of the surrounding soil solutions which (vessels) form a continuous pipe line from the roots to the leaves, for once the water from the soil reaches the main transpiration current in the xylem vessels, water is taken upwards to the leaves for utilisation and for ultimate escape of the excess water through stomata.

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As a result there generally is a higher osmotic pressure in the sap of the xylem vessels than the water outside the root hair in the soil.

The osmotic pressure of the root hair cells generally varies from 3 to 5 atmospheres. Thus whenever water potential of such soil exceeds that of young root cells and root hairs, water will move from soil into the root and since the osmotic pressure of the soil solution in inert soils is only a fraction of an atmosphere, the absorbing capacity or suction need not be very great before water will enter them.

The absorption process described above accounts for the intake of water which the roots absorb. This mechanism of absorption of water is referred to as **passive absorption** because the entry of water into the roots is brought about by conditions which originate in the top of the plant and the root cells apparently play a passive role.

The water can be absorbed by root cells by forces which develop there and are often called **active absorption**. This active absorption apparently takes place in plants when transpiration rate is relatively low and the soil contains water in abundance. This active absorption of water can only be in very small amounts since water would tend to leak out so rapidly by diffusion that enormous amount of respiratory energy would be required to maintain the gradient.

Mechanism of Water Absorption:

The actual absorption of water when a root hair is in contact with a number of cortical cells of the root and xylem vessel, water will enter the root hair, pass from there into the cortical cells and finally into the xylem if there be a gradient of water potential from the root hairs to the xylem vessels.

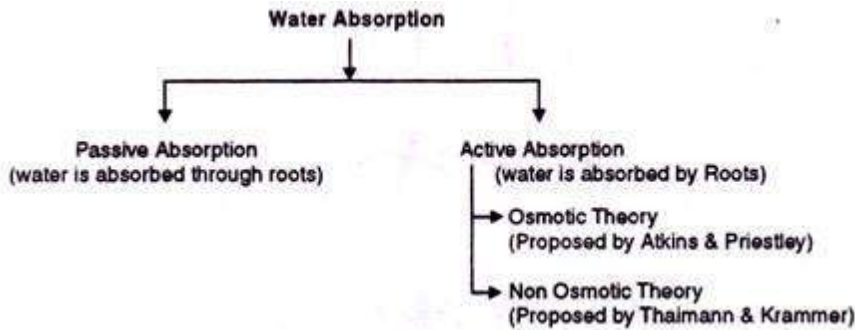
It is the gradient of water potential from the root hair to the xylem vessels that is essential for the absorption of water by the roots.

The mechanism of water absorption can be explained by two approaches.



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1. Passive absorption:

Root does not play active role. Force responsible for absorption develops due to transpiration. No expenditure of energy for absorption, process.

1. As the leaf cells lose water in transpiration, they develop water deficit (turgor deficit, D.P.D. or Suction force)
2. Their water potential becomes lower and they draw water from the xylem of the veins.
3. This causes the xylem of the veins to develop the S.F. as well as low water potential.
4. Therefore, veins draw water from the petiole, the petiole from the stem, and the stem from the root, and hence water from the soil automatically enters the roots through the root hairs.
5. Thus, the suction force responsible for the absorption of water by the root actually originates
6. The root system merely acts as a physical absorbing system.

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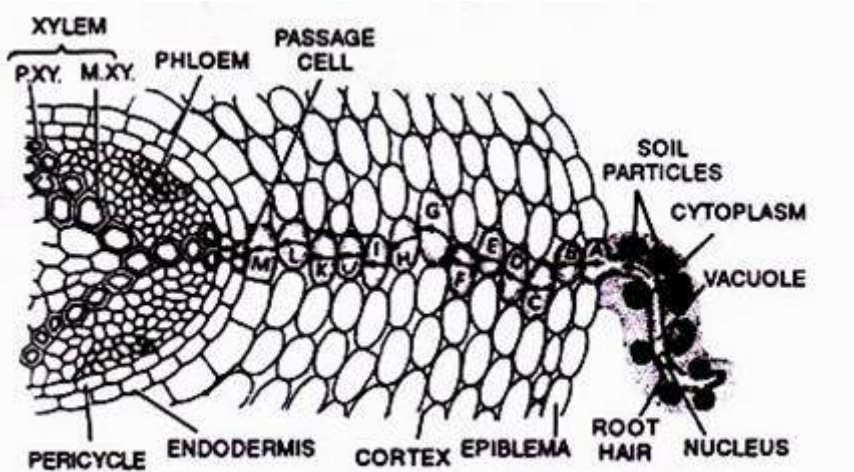


Fig. 2.6 Diagrammatic cross section of root showing pathway of water uptake. (The arrows indicate the direction of movement of water)

2. Active Absorption:

Water is absorbed as a result of activity of root and does not concern with any role of shoot. Two theories have been put forward to explain the actual mechanism of active absorption.

(a) Osmotic theory

(b) Non osmotic theory

(a) Osmotic theory:

Proposed by Atkins and Priestley. Water is absorbed due to osmotic difference between soil water and that of tonoplasm. D.P.D. of root hair is increased due to high O.P and low TP of root water is absorbed by endosmosis TP of root hair increase and D.P.D. decreases water moves from root hair to inner cells and finally reaches into the xylem.

(b) Non Osmotic theory:

Proposed by Thimann and Kramer. Water absorption is an active process occurs due to non osmotic reason against the DPD. Process require energy (ATP) comes from respiration.

1. Operate in very slowly transpiring plants.

2. Occurs against the D.P.D. gradient and requires the expenditure of energy released from respiration.

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3. There may be some carrier substances in the wall of root cells, which bind with water and carry, it to the inner tissue, (certain bacteria in higher plants).

4. Auxin increases rate of the transpiration as well as water absorption.

Root pressure, guttation and bleeding are the manifestation of active water absorption. The available evidence indicates that passive absorption accounts for most of the water absorbed by plants. Active absorption is important only in slowly transpiring plants growing in soil near field capacity.

Ascent of Sap and (Transpiration pull theory)

According to this theory physical forces in xylem elements of plants are responsible for Ascent of sap.

1) Capillary force theory -According to Boehm capillary force in xylem vessel is responsible.

Objection:- Capillary force can not function due to cross wall at each cell, magnitude is low, ends of vessels are not dipped in water, lower terrestrial plants have only tracheids. **2) Imbibitional theory** :-

According to Sachs & Unger Imbibitional force is responsible for Ascent of sap. Objection:- Ascent of sap is through lumen of vessel and not through wall. **3) Atmospheric theory** :- According to this

theory water moves up in xylem to fill up drop in atmospheric due to loss of water during transpiration. Objection:- No vacuum at upper end of plant for atm. Pressure to elevate water beyond 30ft., lower end of the column do not directly open in soil water.

4) Transpiration pull theory :- This theory was proposed by Dixon & Jolley, supported by renner, Curtis, Clark, Levitt. According to this theory two forces are responsible for ascent of sap.

They are 1) Cohesive & Adhesive properties of water to form water column 2) Transpiration pull exerted on this column. water molecules are held together tightly due to strong cohesive force (mutual force of attraction due to hydrogen bonds between them) They also have Adhesive property ie strong attraction between water column and inner walls of xylem. Thus continuous water column is formed from leaf to root which can not be broken.

The water net has two terminals Root tip near absorbing region and sub stomatal cavity in mesophyll. Transpiration creates DPD, results in flow of water from adjacent mesophyll cells, this DPD reaches cells abutting vasculature & xylem elements. Due to continuous transpiration, high DPD causes tension on the water column & it is transported down into root up to area of absorption. Thus water is pulled up due to suction force (Transpiration pull) due to transpiration. Objection :- Entry of air bubbles in the xylem disturb the continuity of water column water column..

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Transpiration: Structure of stomata, Stomatal mechanism (Steward and K- ion theory) Factors affecting transpiration; Anti-transpirants . 6 Hrs

Transpiration takes place through surface of leaves. It is known as Foliar transpiration (more than 90%). Transpiration occurs through young or mature stem is called as Cauline transpiration.

Depending upon the plant surface, transpiration is classified into three types:

1. Stomatal Transpiration:

Water vapour diffuses through minute pore (stomata) present in soft aerial part of plant is known as Stomatal Transpiration. about 85 – 90% of water loosed by the **stomatal transpiration**.

2. Lenticular Transpiration:

Water evaporate through openings present on the older stems called Lenticels and the transpiration that takes place through Lenticel is known as **Lenticular Transpiration**.

Huber observed in some plants that water lost by lenticular transpiration was about 0.1% .

3. Cuticular Transpiration:

Water evaporate through cuticle is called cuticular transpiration. The amount lost is about 5 to 10 percent of the total transpiration. It depends upon the thickness of the cuticle and

Structure of stomata

The stomata are very minute apertures, found on the epidermis of the leaves. Each stoma is surrounded by two kidney-shaped special epidermal cells, known as guard cells.

The epidermal cells surrounding the guard cells of the stoma are known as accessory or subsidiary cells. The number of stomata may range from thousands to lacs per square centimeter on the surface of the leaf.

Each stoma is surrounded by two guard cells. The kidney-shaped guard cells contain chloroplasts.

stomatal mechanism in plant cells.

The mechanism of the closing and opening of the stomata depends upon the presence of sugar and starch in the guard cells.

During day time or in the presence of light, the guard cells of the stomata contain sugar synthesized by their chloroplasts. The sugar is soluble and increases the concentration of the sap of guard cells. Due to higher concentration of the cytoplasm of guard cells, the water comes to them from the neighbouring cells by Endosmosis and they become turgid. With the result the stomata remain open.

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In the night or in the absence of light the sugar present in guard cells converts into the starch. The starch is insoluble, and this way the cell sap of the guard cells remains of much lower concentration than those of neighbouring cells, and the neighbouring cells take out the water from the guard cells by Exosmosis making them flaccid and the stomata closed.

The conversion of sugar into starch during night and vice-versa in day time depends upon the acidity (pH) and alkalinity of the cell sap of guard cells.

During night there is no photosynthesis and the carbon dioxide accumulates in the guard cells, converting the cell sap into weak acidic starch.

During day time the carbon dioxide is used in the process of photosynthesis, the cell sap becomes alkaline and the starch converts into sugar.

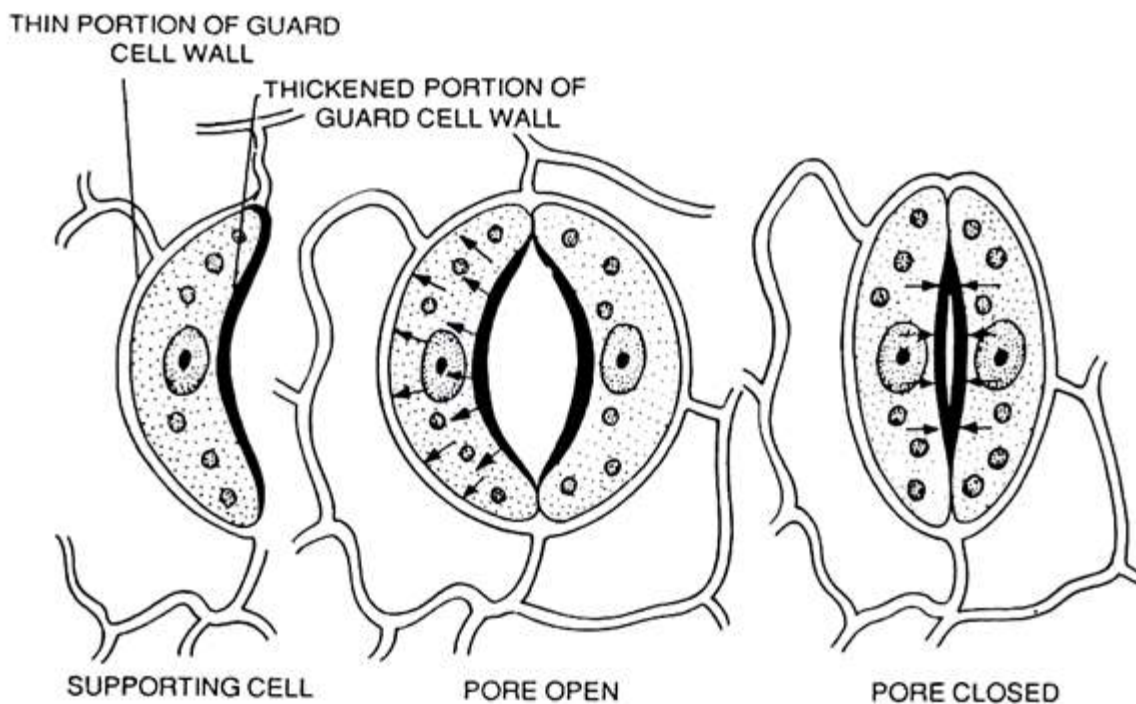


Fig. 2.12. Opening and closing of stomata.

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The important theories of stomatal movement are as follows: Many theories such as Theory of photosynthesis, Theory of glycolate metabolism , Starch Sugar inter-conversion theory , potassium transport ion theory are put forward time to time to explain stomatal mechanism.

Starch Sugar Inter-conversion Theory:

(i) According to **Lloyd** (1908) turgidity of guard cell depends on inter-conversion of starch and sugar. It was supported by **Loft-field** (1921). He found out that guard cells contain sugar during day time when they are open and starch during night when they are closed.



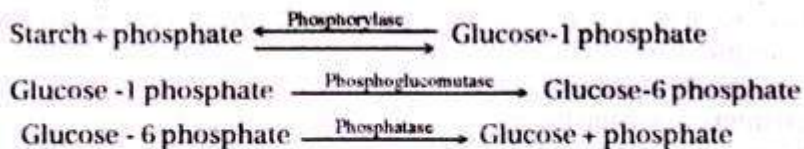
(ii) **Sayre** (1926) observed that during day time due to constant removal of carbon-dioxide by photosynthesis stomata open in neutral or alkaline pH, . Stomata remain closed during night when there is no photosynthesis and due to accumulation of carbon-dioxide, carbonic acid is formed that causes the pH to be acidic. It is supported by **Scarth** (1932) and **Small et. al.** (1942).

iii) **Yin and Tung** (1948) observed that during Day time starch is converted into glucose-1, phosphate in the presence of an enzyme phosphorylase and stomata opens. dark phases (changing CO₂ concentration) control the changes in pH.

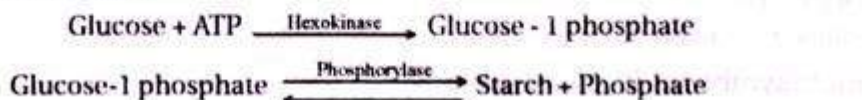
(iv) Steward's scheme:

Steward (1964) proposed modified scheme of inter-conversion of starch and sugar for stomatal movement. According to him conversion of starch to Glucose -1 phosphate is not sufficient. It should be converted to glucose to increase sufficient osmotic pressure. For this, ATP is also required through respiration in presence of oxygen. Guard cell carries enzymes like Phosphorylase, Phosphoglucomutase, Phosphatase and Phosphorylase. These enzymes help in opening and closing of the stomata.

Stomata open :



Stomata close :



Based on the above mentioned theory, process of opening and closing of stomata may be summarized as given below.

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In Light: Photosynthesis takes place

- (1) → Decreased CO₂ Concentration in leaf cells
- (2) → Increase in pH of guard cells
- (3) → Hydrolysis of starch to sugar by enzymes
- (4) → Increase of Osmotic Pressure of guard cells
- (5) → Endosmosis of water in guard cells
- (6) → Increase in T.R of guard cells
- (7) → Aperture opens (Fig. 4.6)

Demerits of the starch-sugar inter-conversion theory:

Demerits of starch-sugar inter-conversion theory are as follows:-

1. In the presence of light when starch disappears from guard cells, malic acid appear and not the sugars.
2. Starch has not been reported in the guard cells of many monocots such as Iris, Amatyllis, Allium.
3. According to this theory O.P. of guard cells increases due to the formation of glucose-1- phosphate in guard cells but it is found that the presence of phosphate ions causes the development of same O.P as does the presence of glucose-phosphate.
4. Enzyme phosphorylase helps in conversion of starch to glucose-1-phosphate but not in the formation of starch from glucose-1-phosphate. This reaction is controlled by some other enzyme about which we do not know yet.

Active K⁺ Transport or Potassium Pump Theory and Role of Absciscic Acid: Or Active Potassium Pump Theory

The concept of K⁺ ion transport was given by **Fujino** and supported ,elaborated by **Levitt & Rashke** in 1975. It is an active mechanism which needs ATP. Mechanism is explained as follows.

Opening of Stomata during Daytime (in presence of light):

Opening of stomata depends upon following conditions:

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- (a) In Presence of light.
- (b) Decrease in starch contents of guard cells.
- (c) Increased concentration of malic acid in guard cells.
- (d) Influx of K^+ ions into guard cells.
- (e) Efflux of H^+ ions from guard cells.
- (f) Intake of Cl^- ions by guard cells.
- (g) Low CO_2 concentration in an around guard cells.
- (h) High pH (more than 7) in guard cells (hence, alkaline medium of the cell sap in guard cells).
- (i) High Turgor Pressure in guard cells due to endosmosis, (turgidity of cells).
- (j) And stomata open.

Explanation of Levitt Concept:This is explained as follows:

In the guard cells, starch is converted into malic acid in presence of light (during day time). Protons (H^+) thus formed are used by the guard cells for the uptake of K^+ ions (in exchange for the protons H^+). This is an active ionic exchange and requires ATP energy and cytokinin (a plant hormone). The concentration of K^+ ions increases and the concentration of H^+ ions decreases in guard cells. The pH of the cell sap in guard cells also increases (pH becomes more than 7 and the medium becomes alkaline).

There is also an increased uptake of Cl^- (anions) by the guard cells to maintain the electrical and ionic balance inside and outside the guard cells. The malate anions formed in the guard cells are neutralized by the K^+ ions. This results in the formation of potassium malate.

Malate anions + K^+ → Potassium malate

Potassium malate enters the cell sap of the guard cells thereby reducing the water potential while increasing the osmotic concentration (and the O.P.) of the cell sap. Hence, endosmosis occurs, guard cells become turgid and kidney-shaped and the stomata opens.

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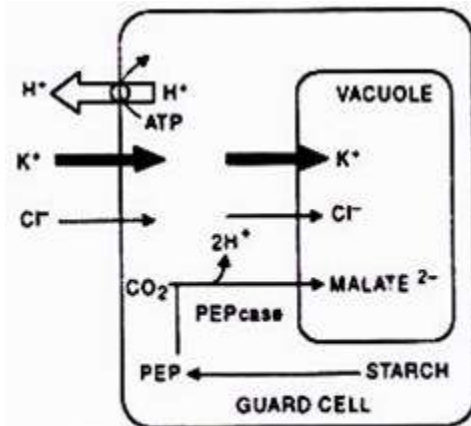


Fig. 4.7 Role of K⁺, Cl⁻ and malate in increasing osmotic concentration (decreasing water potential) of guard cells.

It is also observed that the CO₂ concentration is low in and around guard cells during day time. This is due to high photosynthetic utilization of CO₂. It helps in opening of stomata.

B. Closing of Stomata in Absence of Light (Darkness/Night Time):

Closing of stomata depends on following conditions:

- Absence of light.
- Decreased concentration of malic acid in guard cells.
- Efflux of K⁺ ions from guard cells.
- Influx of H⁺ ions in guard cells.
- Acidic medium of the cell sap in guard cells.
- Loss of Cl⁻ ions from guard cells.
- Increases CO₂ concentration in and around guard cell due to release of CO₂ in respiration combined with the absence of photosynthetic activity in dark.
- Presence of plant growth inhibiting hormone abscissic acid (ABA),
- Loss of turgidity and loss of kidney-shape by guard cells.

All these conditions represent the reversal of the daytime events. Under these conditions, the guard cells lose water by exosmosis and become flaccid. This causes closing of the stomata.

Factors Affecting Transpiration in Plants

1. Humidity of Air:

Humidity or amount of water vapour in the atmosphere, surrounding the plant has influence on Transpiration. On damp foggy atmosphere the rate of transpiration decreases as the outer air remains saturated with water vapour. The less moisture there is in air, the greater will be the rate of transpiration.

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2. Light or Illumination:

The opening and closing of the stomata depend on light. due to absorption of radiant energy and its transformation into heat, temperature of the leaf is raised bringing about an increase in transpiration rates.

3. Temperature:

It increases the rate of transpiration as it hastens transformation of water into water vapour.

4. Wind:

By wind or air current water vapour given off during transpiration is removed; thus saturation of the surrounding air is avoided which otherwise would retard the rate of transpiration. Winds of high however, retard transpiration, because the stomata close up due to high winds. Moreover, winds of high velocity bring about a reduction in temperature which undoubtedly affects transpiration.

5. Atmospheric Pressure:

When atmospheric pressure is high, the rate of transpiration is low.

Plants growing in high altitudes have distinctly lower atmospheric pressures, and those plants have high rates of transpiration, if other environmental factors are not limiting.

6. Soil Factors:

As all necessary water is absorbed from the soil, factors like water content, composition, temperature, concentration of soil solution, etc., indirectly influence the rate of transpiration.

Unit2: Mineral nutrition

Essential elements, macro and micronutrients; Role and deficiency symptoms of Nitrogen, phosphorus, Potassium, Magnesium, Zinc, boron, and Molybdenum Hydroponics 3 Hrs
Mineral Nutrition in Plants:

All green plants are autotrophs. Hence, they require the supply of inorganic materials from outside for synthesis of their own organic material.

Apart from the elements carbon, hydrogen and oxygen that may be absorbed as water, carbon dioxide or oxygen, together makeup a large part of the weight of a plant, all the inorganic materials are absorbed by the plants directly or indirectly from the soil with the help of their roots.

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The source of these inorganic materials in the soil is minerals, they are called as **mineral elements or mineral nutrients**. The process involving the absorption, distribution and utilization of mineral substances by the plants for their growth and development is called **mineral nutrition**.

Mineral Elements in Plants:

On the basis of their effects on plant, mineral elements are generally of two types:

- i. Essential and
- ii. Non-essential.

Only about 17-20 elements are found to be essential. The rest elements are called non-essential without which a plant can survive and reproduce. The non-essential elements may be beneficial or toxic. Beneficial elements improve growth or reduce disease susceptibility without which a plant can still complete its life cycle. For example, Silicon (Si) in grasses, Sodium (Na) in C_4 plants and halophytes. Toxic elements impair growth either in low or high concentrations.

Any mineral ion concentration in tissues that reduce the dry weight of tissues by about 10% is considered toxic. Toxic level for any element also varies for different plants. For example, aluminum (Al) is always toxic in the acidic soil but acts as beneficial element for tea plant.

Na, Zn, B, Mo, Mn, Cu and Fe are toxic if present at high concentration in soil. It is very often seen that the uptake of one element inhibits the uptake of another element. For example, excess of magnesium uptake induce deficiency of iron, magnesium and calcium.

1. Macronutrients Element:

Carbon, Hydrogen and Oxygen:

Although these macronutrients elements are not minerals in the true sense, they are still included in the list as they are most essential for plant life. These three elements are also called framework elements. Plants absorb them from air and soil in the form of carbon dioxide and water.

2. Macronutrients Element:

Nitrogen:

Soil is the chief source of nitrogen. **It is absorbed from the soil in two major ionic forms:**

Nitrate (NO_3^-) and ammonium (NH_4^+). Soils generally remain deficient in nitrogen, and soil fertility always depends on added nitrogen.

Functions of Nitrogen:

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- (i) The most recognized role of nitrogen in the plant is its presence in the structure of protein molecule.
- ii) It is the constituent of such important biomolecules like purines and pyrimidine's which are found in DNA and RNA.
- (iii) Nitrogen is found in the structure of porphyrin molecules which are the precursors of chlorophyll pigments and cytochromes that are essential in photosynthesis and respiration.
- (iv) The coenzymes like NAD^+ , NADP^+ , FAD , etc., are essential to the function of many enzymes and nitrogen is a structural component of these coenzymes.
- (v) Other compounds in the plant such as some vitamins contain nitrogen.

Deficiency Symptoms of Nitrogen:

- (i) A general chlorosis, i.e., the yellowing of leaves, especially in the older leaves, due to a loss in chlorophyll content appears first. In severe cases these leaves become completely yellow and then light tan as they die and frequently fall off the plant.
- (ii) This yellowing symptom appears last in the younger leaves, because they receive soluble forms of nitrogen transported from older leaves.
- (iii) In some cases production and accumulation of anthocyanin pigments is found. As a result a purplish colouration appears in stems, petioles, and lower leaf surfaces.
- (iv) The starch content is increased with the decrease in protein content.

- v) Plant growth remains stunted and lateral buds remain dormant.
- (vi) Flowering is suppressed or delayed; in the latter case the fruits and seeds are small and weak.

3. Macronutrients Element:

Phosphorus:

Phosphorus is very often the limiting nutrient in soils. It is present in the soil in inorganic and organic forms. It is absorbed as inorganic phosphate anions (H_2PO_4^-).

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In the organic portion of the soil organic forms of phosphorus may be found in nucleic acid, phospholipids and inositol phosphates, which are not the utilizable forms of the element. These organic compounds are decomposed, and phosphorus is transformed into an inorganic form which is readily absorbed by the root system.

Factors controlling the availability of phosphorus are:

(i) PH of the soil solution.

(ii) Dissolved aluminum and iron which precipitate out phosphate as un-absorbable aluminum and iron phosphates

(iii) Available calcium which may form salts with all forms of phosphate, that are easily available to the plant due to high solubility in water

(iv) Anion exchange, that takes place between the minerals present in the clay micelles and the phosphate ion under mild acidic conditions

(v) Presence of microorganisms in the soil, which temporarily fix phosphorus in organic structures that is eventually returned to the soil in a bound form for the utilization of plants.

Functions of Phosphorus:

(i) It is a constituent of nucleic acids. Both DNA and RNA have a sugar-phosphate backbone in their structures. Triphosphate forms of nucleotides are precursors of nucleic acids.

(ii) Phosphorus is a constituent of phospholipids or phosphoglycerides or glycerol phosphatides which along with proteins, are characteristic major components of cell membranes.

(iii) Phosphorus is a constituent of the coenzymes NAD^+ and NADP^+ , which take part in most of the cellular oxidation-reduction reactions involving hydrogen transfer. Most of the important metabolic processes like photosynthesis, respiration, nitrogen metabolism, carbohydrate metabolism, fatty-acid metabolism, etc., are dependent on the action of these coenzymes.

(iv) Phosphorus is a constituent of ATP and other high energy compounds.

(v) All the intermediate of glycolysis between glucose and pyruvate are phosphorylated compounds.

Deficiency Symptoms of Phosphorus:

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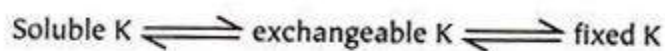
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- (i) Phosphorus-deficient plants may develop dead necrotic areas on the leaves, petioles, or fruits.
- (ii) The plants show a general overall stunted appearance with often dark green colouration.
- (iii) Sometimes phosphorus deficiency may cause leaf-fall and purple or red anthocyanin pigmentation.
- (iv) The older leaves are usually affected first and become dark brown because of the mobility of phosphorus to the younger leaves under deficiency conditions.
- (v) Sometimes distortion in the shape of the leaves is observed and may be confused with zinc deficiency.
- (vi) Large amounts of pith and small amounts of vascular tissues are found in the stems of phosphorus-deficient tomato plants.
- (vii) In some cases a deficiency of this element causes an accumulation of carbohydrates.

Macronutrients Element:

4. Potassium:

Potassium is present in the soil in soluble form, fixed or bound form and in an exchangeable form. Most of the potassium content of the soil is non-exchangeable (fixed) and, unavailable to the plant. Equilibrium exists in the soil between the three forms of potassium.



Functions of Potassium:

(a) Physiological Functions:

- (i) Potassium has been shown to be linked with carbohydrate metabolism.
- (ii) It is essential for translocation of sugar.
- (iii) Stomatal opening in higher plants requires potassium. If there is an influx of potassium ions (K^+) into the guard cells during stomatal opening at the expense of ATP. Potassium accumulation in the guard-cell vacuole results in osmotic swelling of guard cell and stomatal opening.

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(iv) Potassium has a general role in the regulation of water in plant cells. Under water- stress conditions potassium being absorbed selectively prevents the plant from losing water.

(b) Biochemical Functions:

(i) The reactions, involved in the phosphorylation of carboxyl groups and inter-conversions of enol-keto intermediates are activated by potassium.

(ii) Potassium is required by the enzyme acetic thiokinase from spinach leaves for maximal activity.

(iii) Potassium might act as a regulator of the enzyme pyruvate kinase through repression of synthesis of the enzyme.

(iv) Folic acid metabolism has been shown to require potassium.

(v) γ -glutamylcysteine synthesis specifically requires potassium.

(vi) Potassium is required by the enzyme succinyl-CoA synthetase isolated from tobacco for maximal activity.

(vii) Nitrate reductase formation in rice seedlings specifically requires potassium.

(viii) There is an absolute requirement for potassium by starch synthetase isolated from sweet com.

(ix) Potassium, through its role in ATPase activity, may be involved in ion transport across biological membranes.

Deficiency Symptoms of Potassium:

(i) Due to easy mobility of potassium, deficiency symptoms first appear on older leaves. A mottled chlorosis followed by the development of dark necrotic lesions at the tip and margins of the leaf is generally found. The leaf-tips curve downwards and the margins roll inward towards the upper surface.

(ii) In cereals, cells at the leaf-tip and margin die first, and the necrosis spreads basipetally toward the leaf bases.

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(iii) Potassium deficient cereal grains develop weak stalks, and their roots become susceptible to root rotting organisms. As a result, the plants easily get lodged by wind or rain.

(iv) Generally, a potassium deficient plant exhibits stunted growth with shortened internodes.

(v) Anatomically, potassium deficiency causes disintegration of pith cells and formation of secondary pholem in tomato plants.

Macronutrients Element

5. Magnesium:

Magnesium is an exchangeable cation. It is present in the soil in water soluble, exchangeable, and fixed form. Magnesium is found to be present in soil abundantly as magnesium silicate, an unavailable form which becomes available to plants after weathering. Magnesium is absorbed as divalent Mg^{2+} . It may be available to plants from some fixed forms of minerals like magnesite ($MgCO_3$), livine [$(MgFe)_2SiO_4$], and dolomite ($MgCO_3 \cdot CaCO_3$). Of them dolomite is the most popular and economical source of magnesium fertilizer.

Functions of Magnesium:

Magnesium, like calcium, also serves as a structural component and is involved as a cofactor in many enzymatic reactions.

(a) Structural Functions:

(i) Magnesium is a component of the chlorophyll structure.

(ii) Magnesium is required to maintain ribosome integrity.

(iii) Magnesium is necessary to maintain the structural integrity of chromatin fibre. It is involved in coiling of 110Å thick DNA histone protein fibre to form a 300Å thick chromatin fibre.

(b) Physiological and Biochemical Functions:

(i) Magnesium plays two very important roles in plant in photosynthesis and carbohydrate metabolism.

(ii) The release of energy in the hydrolysis of high energy compounds like ATP is greatly influenced by Mg^{2+} . It complexes with ATP, ADP and AMP with differing affinities, resulting in hydrolysis of these compounds.

(iii) Mg^{2+} has also a direct role on potassium-sodium stimulated ATPase activity.

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(iv) Mg^{2+} is necessary for full activity of the two principal CO_2 fixing enzymes, PEP carboxylase and RuBisCO.

(v) Mg^{2+} is also an activator for DNA and RNA polymerases involved in DNA and RNA synthesis from nucleotide triphosphates. Thus Mg^{2+} helps in protein synthesis by activating enzymes of nucleic acid synthesis and forming imitation complexes with mRNA, ribosome and fMet initiator tRNA.

Deficiency Symptoms of Magnesium:

(i) Extensive interveinal chlorosis of the older leaves is the first symptom, and as the deficiency becomes more acute, eventually reaches the younger leaves. This is because magnesium is a mobile element.

(ii) Chlorosis is followed by anthocyanin pigmentation and then necrotic spotting.

(iii) Anatomically magnesium deficiency causes extensive chlorenchyma development and scanty pith formation.

Micronutrients in plant growth.

Some elements are needed for the normal growth of the plant but only in minute amounts. They are essential for the life and the growth of the plants in small amounts, called trace or micronutrient elements.

Trace elements can be conveniently divided into four groups:

(a) **The essential**—so far the following six have been conclusively proved to be essential for normal plant growth—B, Zn, Cu, Mn, Mo and Co

(b) **The probably essential**—elements like selenium, barium, etc.

(c) **The toxic**—all essential macro- and micronutrients in high dosages.

[d) **Physiologically inactive elements**—Arsenic, etc.

1. ZINC

2. BORON

3. MOLYBDENUM

In many parts of the world, economic cropping would have probably ceased but for diagnosis of micro-elemental deficiency and subsequent remedial treatment.

Microelement shortage is not always induced by a real absence of the particular element in the top soil. A large supply may be present but it may be locked up as a result of soil condition and thus unable to enter the soil solution and become available to the roots.

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Atomic Zn is an activator component for a host of enzymes, e.g., carbonic anhydrase (catalyses the reaction $\text{CO}_2 + \text{H}_2\text{O} \rightarrow \text{H}^{++} \text{HCO}_3^-$), alcohol dehydrogenase, lactic dehydrogenase, glutamic dehydrogenase, triosephosphate dehydrogenase, aldolase (can be replaced by Co or Fe), etc.

It has been known for quite a long time that Zn is essential for the formation of the most important plant hormone, indole acetic acid. More recently it was shown that Zn in minute traces is indispensable for the formation of the amino acid, tryptophan, the generally accepted precursor of indole acetic acid and is not directly concerned with the synthesis of the auxin.

The catabolic breakdown of tryptophan to, the indole nucleus of the auxin is accomplished by the enzyme tryptophanase. At the present moment, however, it is clear that the essentiality of Zn for the formation and breakdown of tryptophan is certainly due to its acting as an activator component of enzyme tryptophanase.

- Severe **Zn** deficiency effects on fruit trees.
- Zn salts spray of a very dilute solution of ZnSO_4 on the foliage of the trees.
- Injection of solid salt containing a trace element into the trunk of the tree.

Boron deficiency is usually due to absence of boron in the soil for borates tend to be easily washed out of the soils, the remedy of applying boron to the soil is reasonably effective.

boron forms a complex with sugar in the plant cells that can penetrate through the living cell walls, more rapidly than free sugars and is, therefore, more readily translocated to the growing meristematic cells where the carbohydrates are most needed.

It is now known definitely that **molybdenum** plays an important role in nitrate assimilation in plants and the fixation of atmospheric nitrogen by micro-

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Unit 3: Photosynthesis (10 Hrs)

Photosynthetic apparatus, Photosynthetic Pigments (Chl a, b, xanthophylls, carotene); Photo system I and II, reaction centre, antenna molecules; Electron transport and mechanism of ATP synthesis; C₃, C₄ and CAM pathways of carbon fixation.

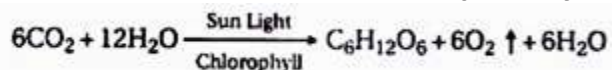
The Process of Photosynthesis in Plants

Introduction:

Photosynthesis (Photon = Light, Synthesis = Putting together) is an anabolic process by which green plant synthesize carbohydrates (initially glucose) using carbon dioxide, water, pigments and sunlight.

Photosynthesis is transformation of solar energy/radiant energy/light energy into chemical energy. According to Van Neil and Robert Hill, oxygen liberated during photosynthesis comes from water and not from carbon dioxide.

Thus, the biochemical reaction for photosynthesis can be written as:



Significance of Photosynthesis:

1. The process of photosynthesis is unique to green and other autotrophic plants. It **synthesizes organic food from inorganic raw materials**.
2. Photosynthesis **converts radiant or solar energy into chemical energy**. The same gets stored in the organic food as bonds between different atoms. Photosynthetic products provide energy to all organisms to carry out their life activities.
3. **Coal, petroleum and natural gas are fossil fuels** which have been produced by the application of heat and compression on the past plant and animal parts (all formed by photosynthesis) in the deeper layers of the earth.
4. All **useful plant products** are derived from the process **of photosynthesis**, e.g., timber, rubber, resins, drugs, oils, fibers, etc.
5. It is the **only known method by which oxygen is added to the atmosphere** to compensate for oxygen being used in the respiration of organisms and burning of organic fuels.

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6. Photosynthesis **decreases the concentration of carbon dioxide** which is being added to the atmosphere by the respiration of organisms and burning of organic fuels.

7. **Productivity of agricultural crops depends upon the rate of photosynthesis.**

Photosynthetic apparatus, Site of Photosynthesis:

All green plant tissues can photosynthesize, but the majority of photosynthesis usually takes place in the leaves. The cells in a middle layer of leaf tissue called the **mesophyll** contain chloroplast.

- **Chloroplast** in green plants constitutes the photosynthetic apparatus and act as site of photosynthesis.
- Chloroplasts of higher plants are **discoid or ellipsoidal** in shape measuring 4 — 6 μ in length and 1—2 μ in thickness.
- It is a **double membranous** cytoplasmic organelle of eukaryotic green plant cells. The thickness of the two membranes including periplastidial space is approximately 300Å.
- Chloroplast is filled with a hydrophilic matrix known as **stroma**. In stroma are embedded a number of flattened membranous sacs known as **Thylakoids**. Photosynthetic pigments occur in thylakoid membranes.
- Aggregation of thylakoids form stacks of coin like structures known as **granna**. **A grannum** consists near about 20 — 30 thylakoids. Each thylakoid encloses a space known **Asloculus**. The end of disc shape thylakoid is called as **margin** and the area where the thylakoids membranes are appressed together is called **partition**. Some of the granna are connected with thylakoids of other granna by **stroma lamella or fret membranes**.
- **In photosynthetic prokaryotes** (blue-green algae and Bacteria) chloroplast is absent. In photosynthetic bacteria **Chromatophore** is present and In blue-green algae **photosynthetic lamellae** are present.

Stroma contains cp-DNA (0.5%), RNA (2—3%), Plastoribosome (70S), enzymes for carbon dioxide assimilation, proteins (50—60%), starch grains and osmophilic droplets, vitamin E and K, Mg, Fe, Mn, P, etc. in traces. **Thylakoid membrane and stroma lamella** both are composed of lipid and proteins.

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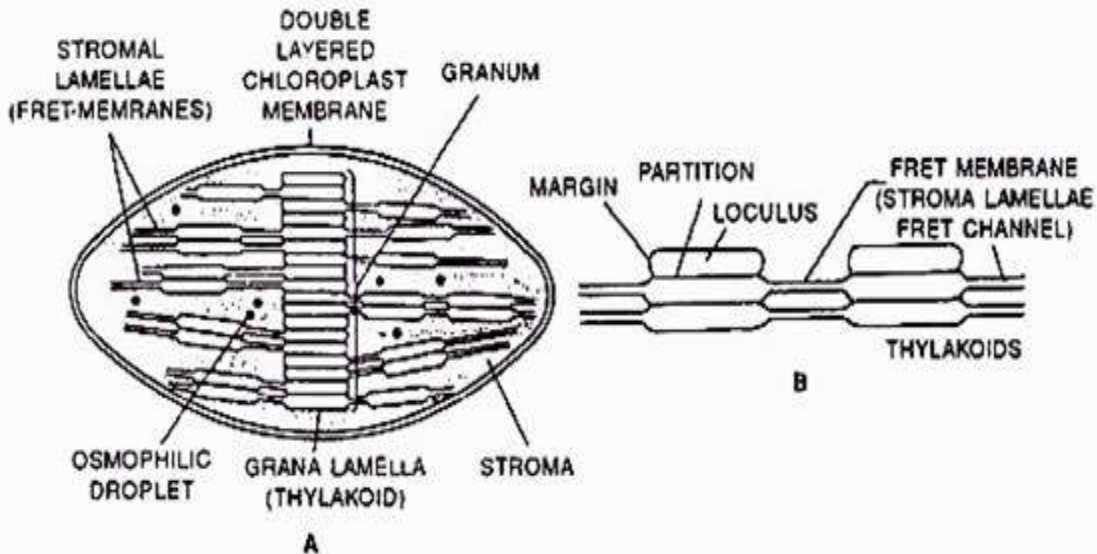


Fig. 6.2. (A) Internal structure of chloroplast (green plastid), (B) Enlarged thylakoids from two grana.

Photosynthetic Pigments (Chl a, b, xanthophylls, carotene)

Photosynthetic pigments are substances that absorb sunlight and initiate the process of photosynthesis.

Photosynthetic pigments are grouped into 3 categories:

(i) Chlorophyll:

These are green coloured most abundant photosynthetic pigments that play a major role during photosynthesis. Major types of chlorophylls which exist in plants and photosynthetic bacteria are **Chlorophyll a, b, c, d and e, Bacteriochlorophyll a, b and g, and Chlorobium chlorophyll.**

The **structure of chlorophyll** was first studied by Wilstatter, Stoll and Fischer in 1912. Chemically a chlorophyll molecule consists of a **porphyrin head** ($15 \times 15\text{\AA}$) and **phytol tail** (20\AA). Porphyrin consists of tetrapyrrole rings and central core of Mg. Phytol tail is side chain of hydrocarbon. It helps the chlorophyll molecules to attach with thylakoid membrane.

Out of various types of chlorophyll, chlorophyll a and chlorophyll b are the most important for photosynthetic process. **Chlorophyll a** is found in all photosynthetic plants except photosynthetic bacteria. Hence it is called as **Universal Photosynthetic Pigment**.

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(ii) Carotenoids:

Carotenoids are **yellow, red or orange colour pigments** embedded in thylakoid membrane in association with chlorophylls but their amount is less. These are insoluble in water and precursor of Vitamin A. These are of two of types, namely Carotene and Xanthophyll .

Carotenes($C_{40}H_{56}$) are pure hydrocarbons, red or orange in colour. Some of the common carotenes are $-\alpha$, β , γ and δ carotenes, Phytotene, Neurosporene, Lycopene (Red pigment found in ripe tomato). β -carotene on hydrolysis gives Vitamin A.

Xanthophylls are yellow coloured oxygen containing carotenoids and are most abundant in nature. The most common xanthophyll in green plant is Lutein ($C_{40}H_{56}O_2$) and it is responsible for yellow colour in autumn foliage. Both carotene and xanthophylls are soluble in organic solvents like chloroform, ethyl ether, carbondisulphide etc.

(iii) Phycobilins (Biliproteins):

These are water soluble pigments and are abundantly present in algae, and also found in higher plants. There are two important types of phycobilins-Phycoerythrin (Red) and Phycocyanin (Blue). Like chlorophyll, these pigments are open tetrapyrrole but do not contain Mg and Phytol chain.

Principles of Light absorption:-

- The source of light for photosynthesis is sunlight. Discrete particles present in light are called photons. They carry energy and the energy contained in a photon is termed as quantum.
- The energy content of a quantum is related to its wave length. Shorter the wave length, the greater is the energy present in its quantum.
- Depending upon the wave length electromagnetic spectrum comprises cosmic rays, gamma rays, X-rays, -UV rays, visible spectrum, infra red rays, electric rays and radio waves.
- Photosynthetic pigments absorb light only in the visible spectrum ranges from 390 nm to 760 nm (3900 – 7600A).
- A ray of light falling upon a leaf behaves in 3 different ways. The leaves absorb near about 83% of light, transmit 5% and reflect 12%. From the total absorption, 4% light is absorbed by the chlorophyll.

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- Visible spectrum can be resolved into light of different colours i.e., violet (390-430 nm), blue or indigo (430-470 nm), blue green (470-500 nm), green (500 – 580 nm), yellow (580 – 600 nm), orange (600 – 650 nm), orange red (650 – 660 nm) and red (660 – 760 nm). Red light above 700 nm is called far red. Radiation shorter than violet are UV rays (100 – 390 nm). Radiation longer than those of red are called infra red (760 – 10,000 nm).

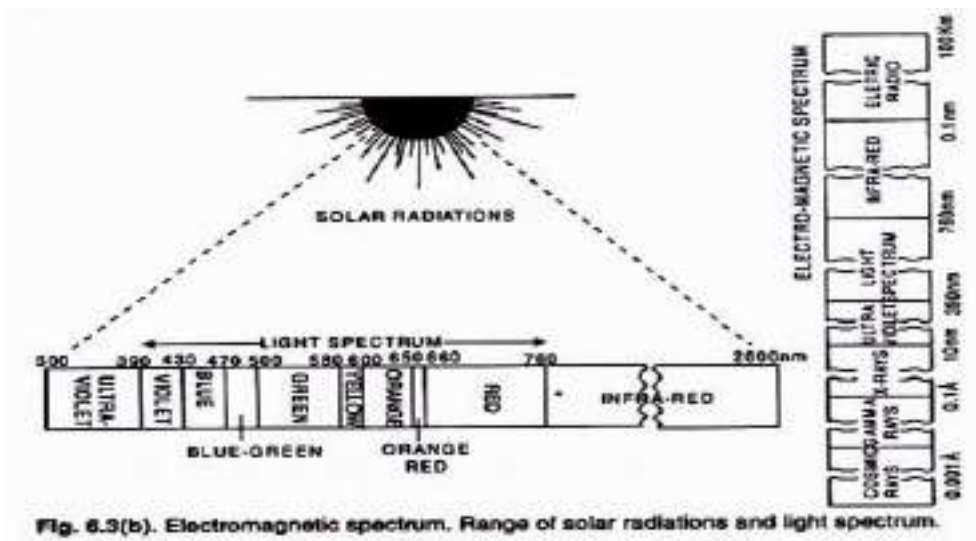


Fig. 6.3(b). Electromagnetic spectrum. Range of solar radiations and light spectrum.

Absorption Spectrum:

All photosynthetic organisms contain one or more organic pigments capable of absorbing visible radiation which will initiate the photochemical reactions of photosynthesis. It varies from pigment to pigment. When the amount of light absorbed by a pigment is plotted as a function of wave length, we obtain absorption spectrum as follows:-

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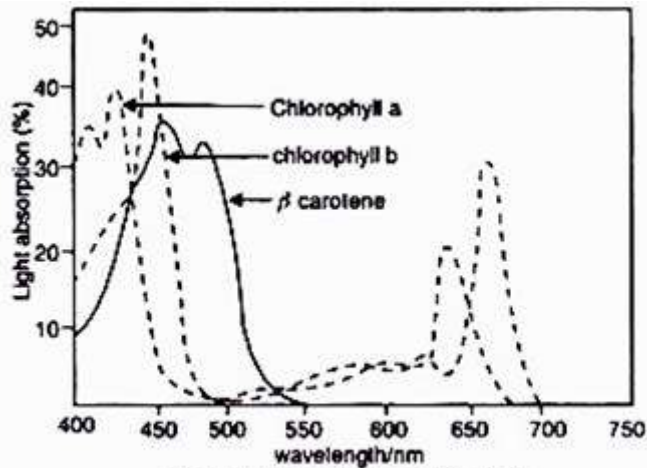


Fig. 6.4 Absorption spectrum of various plant pigments.

Photo system OR Pigment system(PS):-

A group of co-ordinating photosynthetic molecules necessary to affect a photochemical act (absorption and transfer of the light quantum to trapping centre) is called Photo system. R. Emerson suggested the existence of photo system. park and Beggins named the photosystem as 'Quantosomes'.

According to Emerson there are 2 kinds of Photo systems on Grana lamellae and Stroma lamellae. They are Pigment system I (Photosystem I) and Pigment system II (Photosystem II).

Photo system I :-It is the smaller Photo system having size of 110\AA , present on outer surfaces of both Grana lamellae and Stroma lamellae of the chloroplast. It is composed of **Pigment molecules** and **Electron carriers**.

Pigment molecules are chlorophyll and Carotenoids. About 200 Chl-a molecules are present which includes Chl-a₆₇₀, Chl-a₆₈₀, Chl-a₆₉₀ . Single molecule of Chl-a₇₀₀ constitutes **Reaction centre**. Lighth energy from other pigments is transferred to Reaction centre for its conversion. Energy rich electrons are expelled from it for further photochemical act. In addition to it Chl-b and 50 molecules of Carotene are present.

Electron carriers are 1 molecule of **Ferrodixin reducing substance**, 1 or 2 molecules of **Ferrodixin**, 2 molecules of **Cytochrome-b₆** , 1 molecule of **Cytochrome f**, one molecule of **Plastocyanin**.

Role of Photo system I :-

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- 1.** Photo system takes part in trapping light energy and it's conversion into chemical energy.
- 2.** Photo system involves in Cyclic and Non-cyclic Photophosphorylation during Dark reaction.
- 3.** Photo system take part in production of assimilatory powers $\text{NADP}^{++} + \text{H}^+$ and ATP molecules.

Photo system II :-Photo system II is the larger Photo system having size 185\AA . It is located on the inner surface of Grana lamellae. . It is composed of **Pigment molecules** and **Electron carriers**.

Pigment molecules include about 250 molecules of Chl-a, Chl-b, 50 molecules of carotenoids mostly xanthophylls. Chl-a include Chl-a₆₆₀ , Chl-a₆₇₀. Single molecule of Chl-a₆₈₀ or P₆₈₀. It constitutes **Reaction centre**.

Electron carriers are one molecule of **Pheophytin (colourless chlorophyll that laks Mg^{++})**, One molecule of **Plastoquinone**, 2 molecules of **Cytochrome b₆**, unknown protein '**Zn**' and **Mn⁺⁺**.

Role of Photo system II :-

1. Photo system II takes part in trapping light energy and its conversion into chemical energy.
2. Photo system II involves in only Non-cyclic phosphorylation.
3. Photo system II involves in photo ionization of water and liberation of Oxygen.
4. Photo system II takes part in production of assimilatory power ATP molecule.

Both the pigment systems are believed to be **inter-connected by a third integral protein complex** called cytochrome b – f complex. The other intermediate components of electron transport chain viz., PQ (plasto quinone) and PC (plastocyanin) act as mobile electron carriers between two pigment systems

	<u>Photo system II :- PS-I</u>	<u>Photo system II :-PS-I</u>
1	PSI is found in thylakoid membrane and stroma lamella.	PS II is found in thylakoid membrane .
2	It contains pigments chlorophyll a ₆₆₀ , chlorophyll a ₆₇₀ , chlorophyll a ₆₈₀ , chlorophyll a ₆₉₀ , and chlorophyll a ₇₀₀ .	It contains pigments as chlorophyll b ₆₅₀ , chlorophyll a ₆₆₀ , chlorophyll a ₆₇₀ , chlorophyll a ₆₇₈ , chlorophyll a _{680 – 690} and phycobillins.

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3	Chlorophyll a 700 or P ₇₀₀ is the reaction centre of PS I.	P ₆₈₀₋₆₉₀ is the reaction centre of PS II.
4	Chlorophyll a content is more in PS I	Chlorophyll a content is less in PS II
5	Carotenoids are present both in PS I	Carotenoids are present both in PS II
6	PS I is active in both red and far red light.	PS II is inactive in far red light.
7	PS I is associated with both cyclic and non-cyclic photophosphorylation	PS II is associated with only non-cyclic photophosphorylation.
8	wave length longer than 680 nm affect only pigment system I.	Wave length of light shorter than 680 nm affect both the pigment systems

Antenna molecules : Each pigment system consists of a central core complex and light harvesting complex (LHC). LHC comprises **antenna pigments** associated with proteins (antenna complex). Their main function is to harvest light energy and transfer it to their respective reaction centre. The core complex consists of reaction centre associated with proteins and also electron donors and acceptors.

Mechanism of Photosynthesis:-

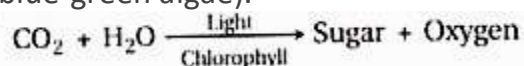
Photosynthesis is an oxidation reduction process in which water is oxidized and carbon dioxide is reduced to carbohydrate.

Blackmann (1905) pointed out that the process of photosynthesis consists of two phases:

(1) **Light reaction** or Light-dependent phase or photochemical phase:- During light reaction, oxygen is evolved and assimilatory power (ATP and NADPH₂) are formed.

(2) **Dark reaction** or Light independent phase or Biochemical phase:-During dark reaction assimilatory power is utilized to synthesize glucose.

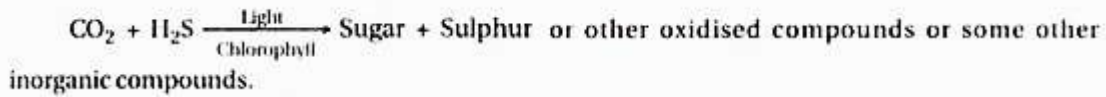
(i) Oxygenic photosynthesis (with evolution of O₂) takes place in green eukaryotes and cyanobacteria (blue-green algae).



(ii) Anoxygenic photosynthesis (without the evolution of O₂) takes place in photosynthetic bacteria.

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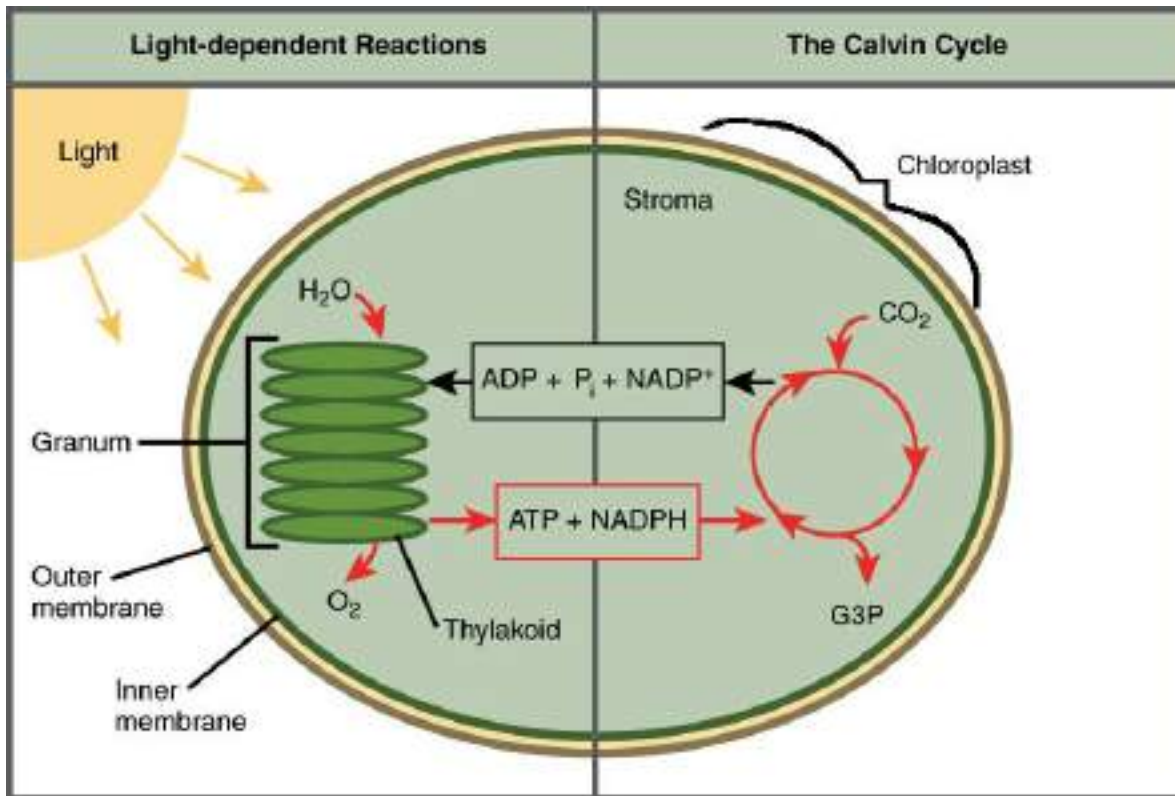


The light-dependent reactions

Photosynthesis in the leaves of plants involves many steps, but it can be divided into two stages: the [light-dependent reactions](#) and the [Calvin cycle](#).

- The **light-dependent reactions** take place in the thylakoid membrane and require a continuous supply of light energy. Chlorophylls absorb this light energy, which is converted into chemical energy through the formation of two compounds, ATP (Adenosine Triphosphate), an energy storage molecule—and NADPH (Nicotinamide Adenine Dinucleotide Phosphate), a reduced (electron-bearing) electron carrier. In this process, water molecules are also converted to oxygen gas—the oxygen we breathe!
- The **Calvin cycle**, also called the **light-independent reactions**, takes place in the stroma and does not directly require light. Instead, the Calvin cycle uses ATP and NADPH from the light-dependent reactions to fix carbon dioxide and produce three-carbon sugars—glyceraldehyde-3-phosphate, or G3P, molecules—which join up to form glucose.

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Schematic of the light-dependent reactions and Calvin cycle and how they're connected.

The light-dependent reactions take place in the thylakoid membrane. They require light, and their net effect is to convert water molecules into oxygen, while producing ATP molecules—from ADP and P_i —and NADPH molecules—via reduction of $NADP^+$.

ATP and NADPH are produced on the stroma side of the thylakoid membrane, where they can be used by the Calvin cycle.

The Calvin cycle takes place in the stroma and uses the ATP and NADPH from the light-dependent reactions to fix carbon dioxide, producing three-carbon sugars—glyceraldehyde-3-phosphate, or G3P, molecules.

The Calvin cycle converts ATP to ADP and P_i , and it converts NADPH to $NADP^+$. The ADP, P_i , and $NADP^+$ can be reused as substrates in the light reactions.

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Quantum Requirement and Quantum Yield:

The solar light comes to earth in the form of small packets of energy known as photons. The energy associated with each photon is called Quantum. Thus, requirement of solar light by a plant is measured in terms of number of photons or quanta.

The number of photons or quanta required by a plant or leaf to release one molecule of oxygen during photosynthesis is called quantum requirement. It has been observed that in most of the cases the quantum requirement is 8.

It means that 8 photons or quantum's are required to release one molecule of oxygen. The number of oxygen molecules released per photon of light during photosynthesis is called Quantum yield. If the quantum requirement is 8 then quantum yield will be 0.125 (1/8).

Photosynthetic Unit or Quantasome:

It is defined as the smallest group of collaborating pigment molecules necessary to affect a photochemical act i.e., absorption and migration of a light quantum to trapping centre where it promotes the release of an electron.

Emmerson and Arnold (1932) on the basis of certain experiments assumed that about 250 chlorophyll molecules are required to fix one molecule of carbon dioxide in photosynthesis. This number of chlorophyll molecules was called the chlorophyll unit but the name was subsequently changed to photosynthetic unit and later it was designated as Quantasome by Park and Biggins (1964).

The size of a quantasome is about 18 x 16 x 10nm and found in the membrane of thylakoids. Each quantasome consists of 200 – 240 chlorophyll (160 Chlorophyll a and 70 – 80 Chlorophyll b), 48 carotenoids, 46 quinone, 116 phospholipids, 144 diagalactosyl diglyceride, 346 monogalactosyl diglyceride, 48 sulpholipids, some sterols and special chlorophyll molecules (P₆₈₀ and P₇₀₀).

'P' is pigment, 680 and 700 denotes the wave length of light these molecule absorb. P₆₈₀ and P₇₀₀ constitute the reaction centre or photo centre. Other accessory pigments and chlorophyll molecules are light gatherers or antenna molecules. It capture solar energy and transfer it to the reaction centre by resonance transfer or inductive resonance.

Photoluminescence:

It is the phenomenon of re-radiation of absorbed energy. It is of two types:

(1) Fluorescence and

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(2) Phosphorescence.

Emerson Red Drop Effect and Enhancement Effect:

R. Emerson and Lewis (1943) while determining the quantum yield of photosynthesis in *Chlorella* by using monochromatic light of different wave lengths noticed a sharp decrease in quantum yield at wave length greater than 680 m μ . This decrease in quantum yield took place in the far red part of the spectrum i.e., the curve shows quantum yield drops dramatically in the region above 680 nm (Red region). This decline in photosynthesis is called Red drop effect (Emerson's first experiment).

Emerson and his co-workers (1957) found that the inefficient far red light in *Chlorella* beyond 680nm could be made fully efficient if supplemented with light of short wave length. The quantum yield from the two combined beams was found to be greater than the effect of both beams when used separately. This enhancement of photosynthesis is called Emerson Enhancement Effect (Emerson's second experiment) (Fig. 6.6).

Rate of oxygen evolution in combined beam – Rate of oxygen evolution in red beam / Rate of oxygen evolution in far red beam

E = Emerson effect.

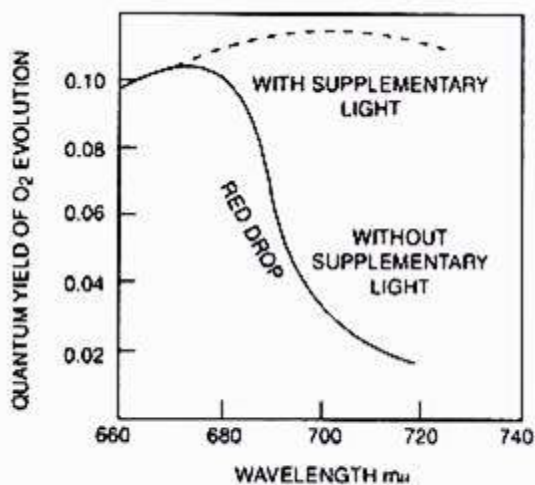


Fig. 6.6 Red drop effect and Emerson's enhancement effect.

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I. Light Reaction (Photochemical Phase):

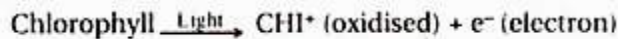
Light reaction or photochemical reaction takes place in thylakoid membrane or granum and it is completely dependent upon the light. The raw materials for this reaction are pigments, water and sunlight.

It can be discussed in the following three steps:

1. Excitation of chlorophyll
2. Photolysis of water
3. Photophosphorylation

1. Excitation of Chlorophyll:

It is the first step of light reaction. When P₆₈₀ or P₇₀₀ (special type of chlorophyll a) of two pigment systems receives quantum of light then it becomes excited and releases electrons. (When photon of light strikes pigment molecules, all the pigments absorb light energy and transfer it to reaction centre Chl-a₇₀₀ PS-I and Chl-a₆₈₀ in PS-II. These chlorophyll molecules become excited and expel energy rich electrons to electron acceptors.

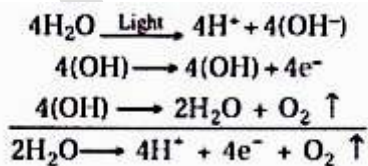


2. Photolysis of Water and Oxygen Evolution (Hill Reaction):

First time Van Neil discovered that the source of oxygen evolution H₂O.

The idea was supported by R. Hill.

The splitting of water into Hydrogen and ions and hydroxyl ions during photosynthesis is called **Photolysis of water**. Mn, Ca, and Cl ions play prominent role in the photolysis of water. Thus formed hydroxyl ions unite to form water, Oxygen and Electrons. This reaction is also known as **Hill reaction**. To release one molecule of oxygen, two molecules of water are required.



The evolution of oxygen from water was also confirmed by Ruben, Randall, Hassid and Kamen (1941) using heavy isotope (O₁₈) in green alga Chlorella.

3. Photophosphorylation:

Synthesis of ATP from ADP and inorganic phosphate (pi) in presence of light in chloroplast is known as **Photophosphorylation**. It was discovered by Arnon (1954).

Photophosphorylation is of two types.

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(a) Cyclic photophosphorylation

(b) Non-cyclic photophosphorylation.

(a) Cyclic Photophosphorylation:

The process of photophosphorylation in which an electron expelled by the excited photo Centre (PSI) is returned to it after passing through a series of electron carriers is called “**Cyclic Photophosphorylation**”.

1. It occurs in low light intensity, wavelength longer than 680 nm and when CO₂ fixation is inhibited.
2. Absence of CO₂ fixation results in non requirement of electrons as NADPH₂ is not being oxidized to NADP⁺.
3. Cyclic Photophosphorylation is performed by photosystem I only. Its photo Centre P₇₀₀ extrudes an electron with a gain of 23 kcal/mole of energy after absorbing a photon of light.
4. After losing the electron the photo Centre becomes oxidized. The expelled electron passes through a series of carriers FeS, ferredoxin, plastoquinone, cytochrome b- f complex and plastocyanin before returning to photo Centre.
5. While passing between ferredoxin and plastoquinone and/or over the cytochrome complex, the electron loses sufficient energy to form ATP from ADP and inorganic phosphate.
6. Halobacteria(Halophile bacteria) also perform Photophosphorylation but ATP thus produced is not used in synthesis of food. These bacteria possess purple pigment bacteriorhodopsin attached to plasma membrane. As light falls on the pigment, it creates a proton pump which is used in ATP synthesis.

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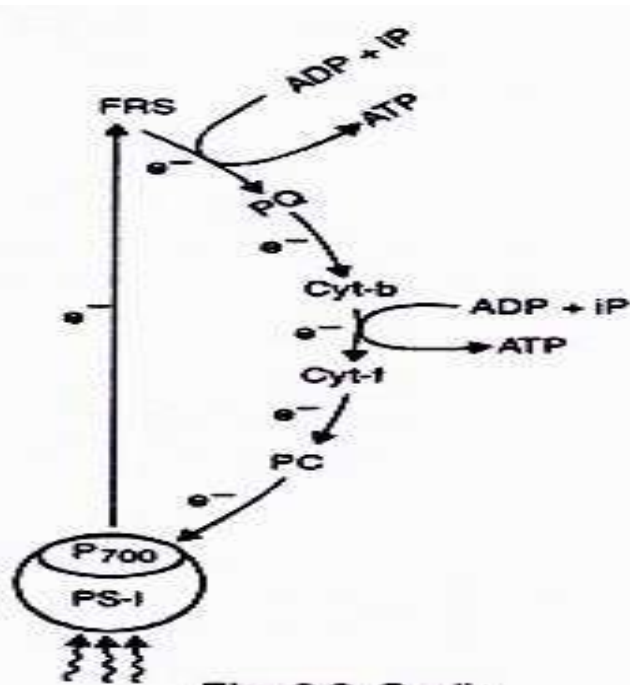


Fig. 6.8 Cyclic photophosphorylation

(b) Noncyclic Photophosphorylation (Z-Scheme):

The normal process of photophosphorylation in which the electron expelled by the excited photo Centre (reaction centre) does not return to it is called "**Noncyclic Photophosphorylation**", It is also called "**Z scheme**" as electrons travel in Zig-Zag manner through electrons carriers.

1. Non-cyclic photophosphorylation is carried out in collaboration of both photo system I and II.
2. Electron released during photolysis of water is picked up by reaction centre of PS-II, called P₆₈₀. When the reaction centre absorbs light energy, these electrons are extruded out. (The extruded electron has an energy equivalent to 23 kcal/mole).
3. It passes through a series of electron carriers— Phaeophytin, PQ, cytochrome b- f complex and plastocyanin.
4. While passing over cytochrome complex, the electron loses sufficient energy for the synthesis of ATP.

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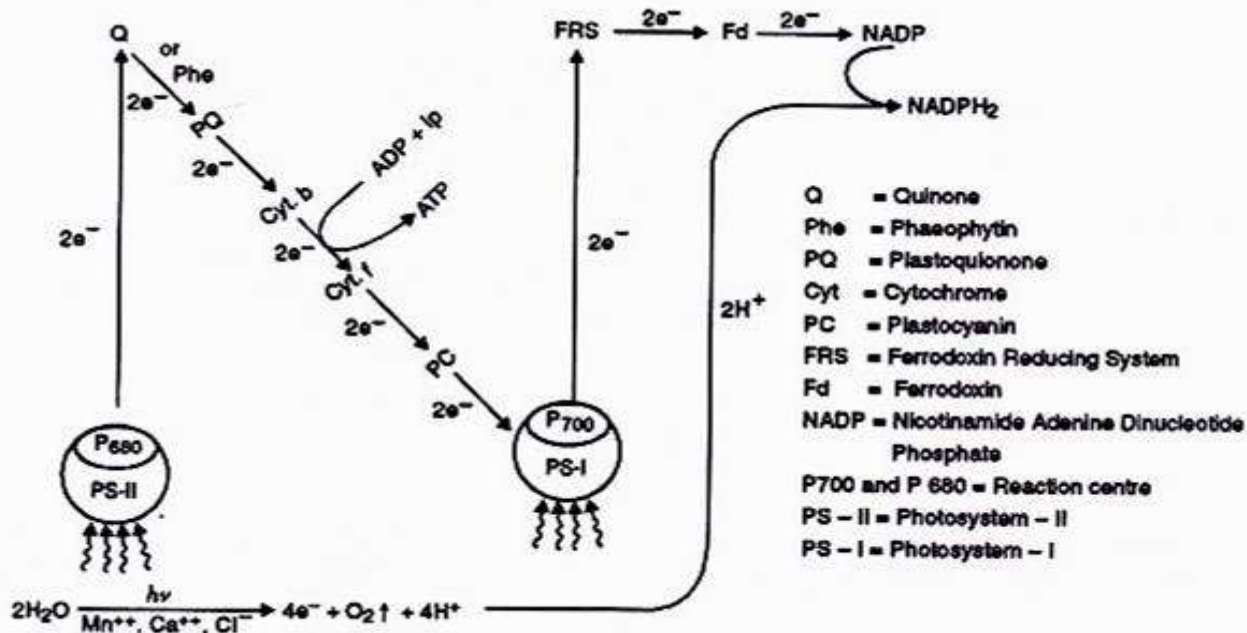


Fig. 6.9. Non-cyclic photophosphorylation and electron transport during photochemical phase.

- The electron is handed over to reaction centre P_{700} of PS-I by plastocyanin. P_{700} extrudes the electron after absorbing light energy.
- The extruded electron passes through FRS ferredoxin, and NADP -reductase which combines it with NADP^+ for becoming reduced through H^+ releasing during photolysis to form NADPH_2 . ATP synthesis is not direct.
- The energy released by electron is used for pumping H^+ ions across the thylakoid membrane. It creates a proton gradient. This gradient triggers the coupling factor to synthesize ATP from ADP and inorganic phosphate (Pi).

CONCLUSION: At the end of Light reaction assimilatory powers like strong reducing agents NADPH_2 and High chemical energy compound ATP are formed with the liberation of Oxygen.

II. Dark Reaction OR Thermochemical reaction OR Carbon assimilation reaction.

Dark reaction is the Second Part of mechanism of photosynthesis. It is not light dependent process. It takes part in Stroma part of the chloroplast. It is an **independent of light**. Hence it is called **Dark reaction**, (But it depends upon the products of light reaction of photosynthesis, i.e., NADPH_2 and

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ATP). In 1905 Blackman demonstrated Thermo chemical reduction of CO_2 into carbohydrate. Hence it is also called “Blackmann reaction”.

The CO_2 assimilation takes place both in light and darkness when the substrates NADPH_2 and ATP are available. CO_2 fixation is closely linked to the light reactions. During evolution three different ecological variants have evolved with different CO_2 incorporation mechanism: **C_3 , C_4 and CAM plants.**

Calvin or C_3 Cycle or PCR (Photosynthetic Carbon Reduction Cycle):

It is the basic mechanism of CO_2 is fixation to form carbohydrates. It was proposed by Melvin Calvin. Calvin along with A.A. Benson, J. Bassham used radioactive isotope of carbon (C^{14}) in *Chlorella pyrenoidosa* and *Scenedesmus* to determine the sequences of dark reaction. For this work Calvin was awarded Nobel Prize in 1961. Hence it is called “Calvin cycle”. During Calvin cycle 3 Carbon compound Phospho glyceric acid is first stable product, hence this path way is also called “ **C_3 cycle**”. To synthesize one glucose molecule Calvin cycle requires 6CO_2 , 18 ATP and 12 NADPH_2 .

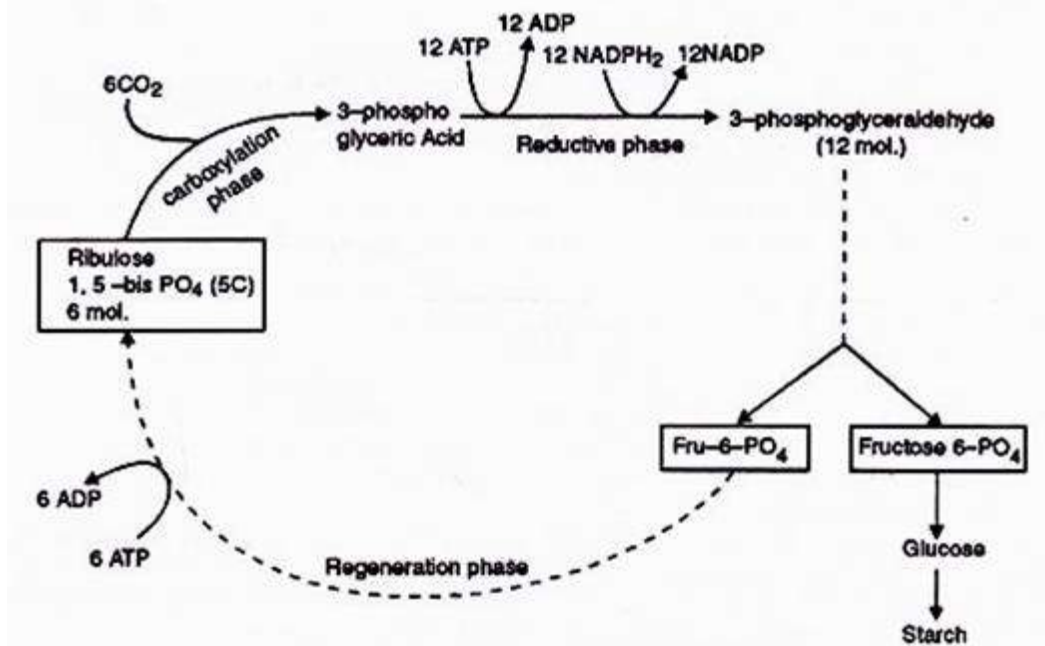


Fig. 6.11 Outline schematic cycle of CALVIN.

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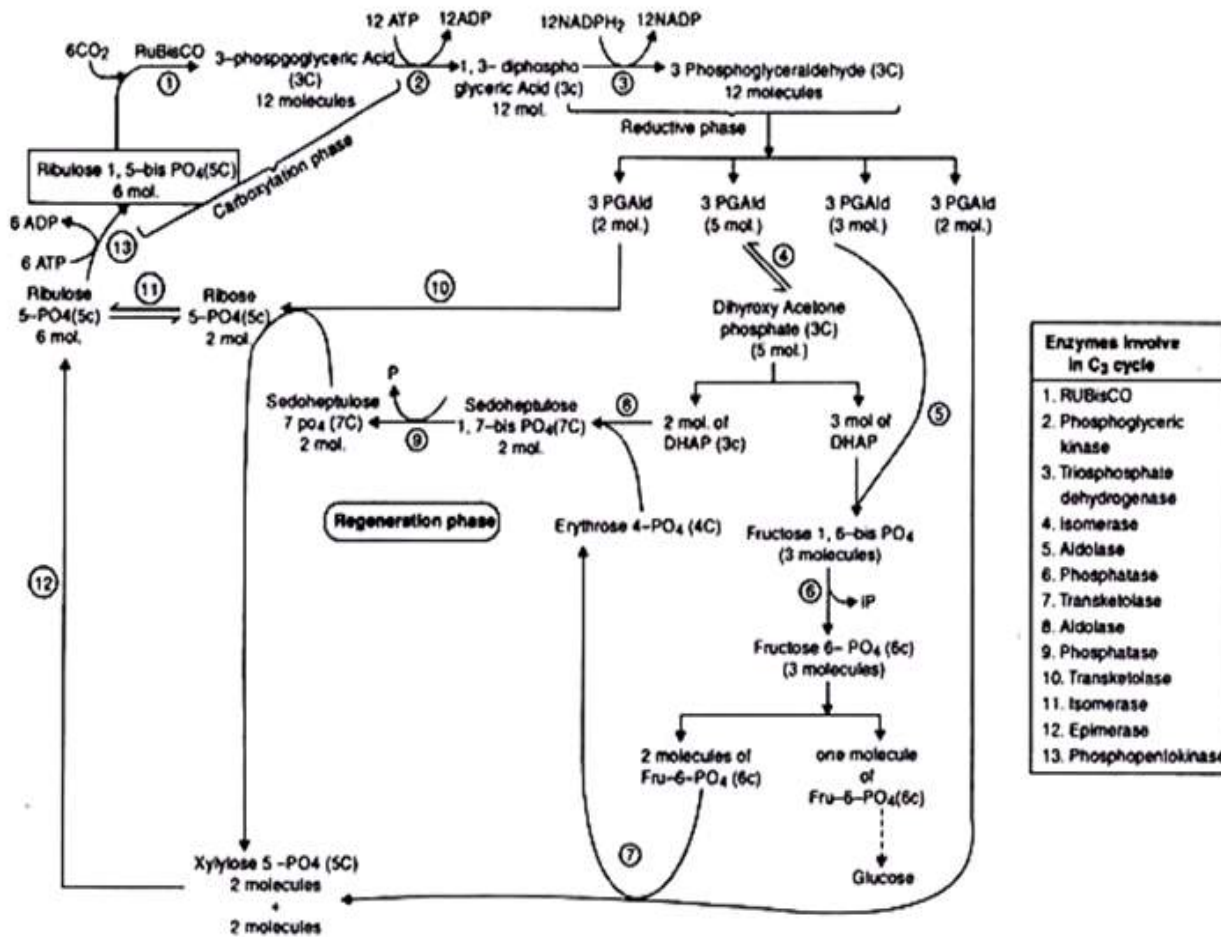


Fig. 6.12 C₃ cycle/CALVIN CYCLE.

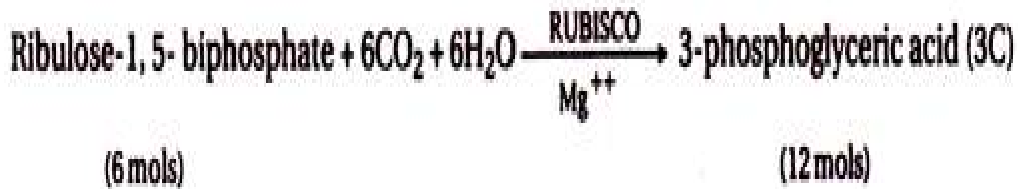
Calvin cycle completes in 4 major phases:

1. Carboxylation phase
2. Reductive phase
3. Glycolytic reversal phase (sugar formation phase)
4. Regeneration phase

1. Carboxylation phase:

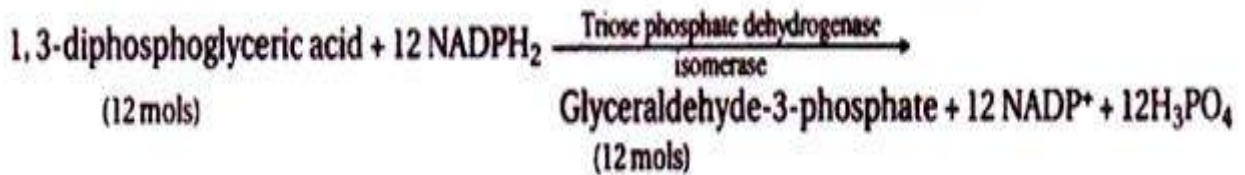
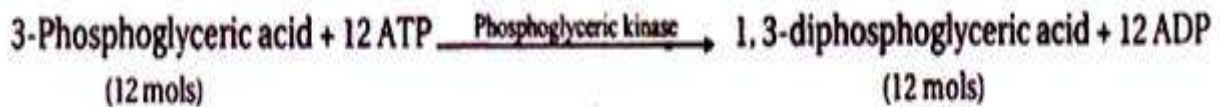
CO₂ enters the leaf through stomata. In mesophyll cells, CO₂ combines with a phosphorylated 5-carbon sugar, called **Ribulose biphosphate** (or RuBP) in presence of an enzyme RUBISCO (RUBP carboxylase). 6 molecules of RUBP absorb 6 molecules of CO₂ to form 6 molecules of unstable Carbon compound. (2-carboxy 3-keto 1,5-biphosphoribitol) Which breaks down into two molecules of first stable product 3-phosphoglyceric acid (PGA) of dark reaction (C₃ Cycle).

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2. Reductive Phase:

The PGA molecules are phosphorylated by ATP molecule and reduced to form 3-phosphoglyceraldehyde (PGAL) by NADPH_2 (product of light reaction known as assimilatory



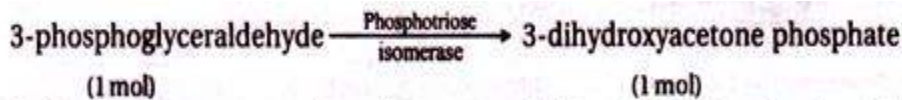
power).

3. Formation of sugar (Glycolytic Reversal Phase):

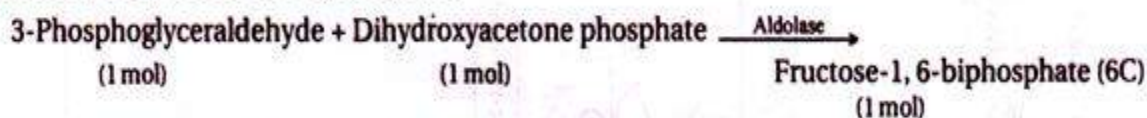
- Out of two molecules of 3-phosphoglyceraldehyde (PGAL) one molecule is converted to its isomer 3-dihydroxyacetone phosphate (DHAP).
- DHAP reacts with one molecule of Phosphoglyceraldehyde to form Fructose 1,6-biphosphate in presence of Aldolase..
- Fructose 1,6-biphosphate is converted into Fructose-6- phosphate and one molecule of phosphoric acid in presence of phosphotase.
- Fructose-6- phosphate is converted into Fructose-1- phosphate which forms Glucose 1- phosphate.
- Fructose-1- phosphate and Glucose 1- phosphate condense to form Sucrose.

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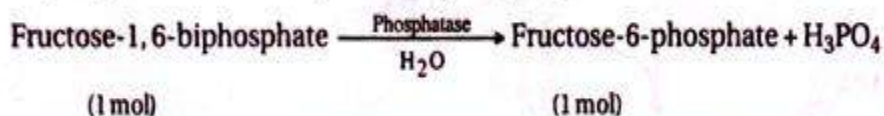
Plant physiology and Metabolism



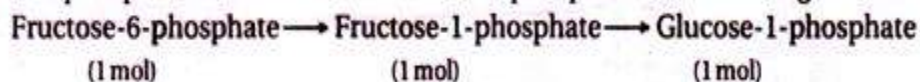
One mol of 3-dihydroxyacetone phosphate reacts with 1 mol of 3-phosphoglyceraldehyde to form one molecule of fructose-1, 6-biphosphate.



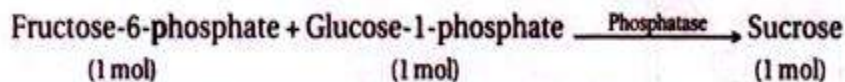
One mol of fructose-6-phosphate and one mol of phosphoric acid is released from one mol of fructose-1, 6-biphosphate with the help of the enzyme phosphatase with utilization of one mol of H₂O.



Fructose-6-phosphate is converted to fructose-1-phosphate which forms glucose-1-phosphate.



Fructose-6-phosphate and glucose-1-phosphate condense to form sucrose.



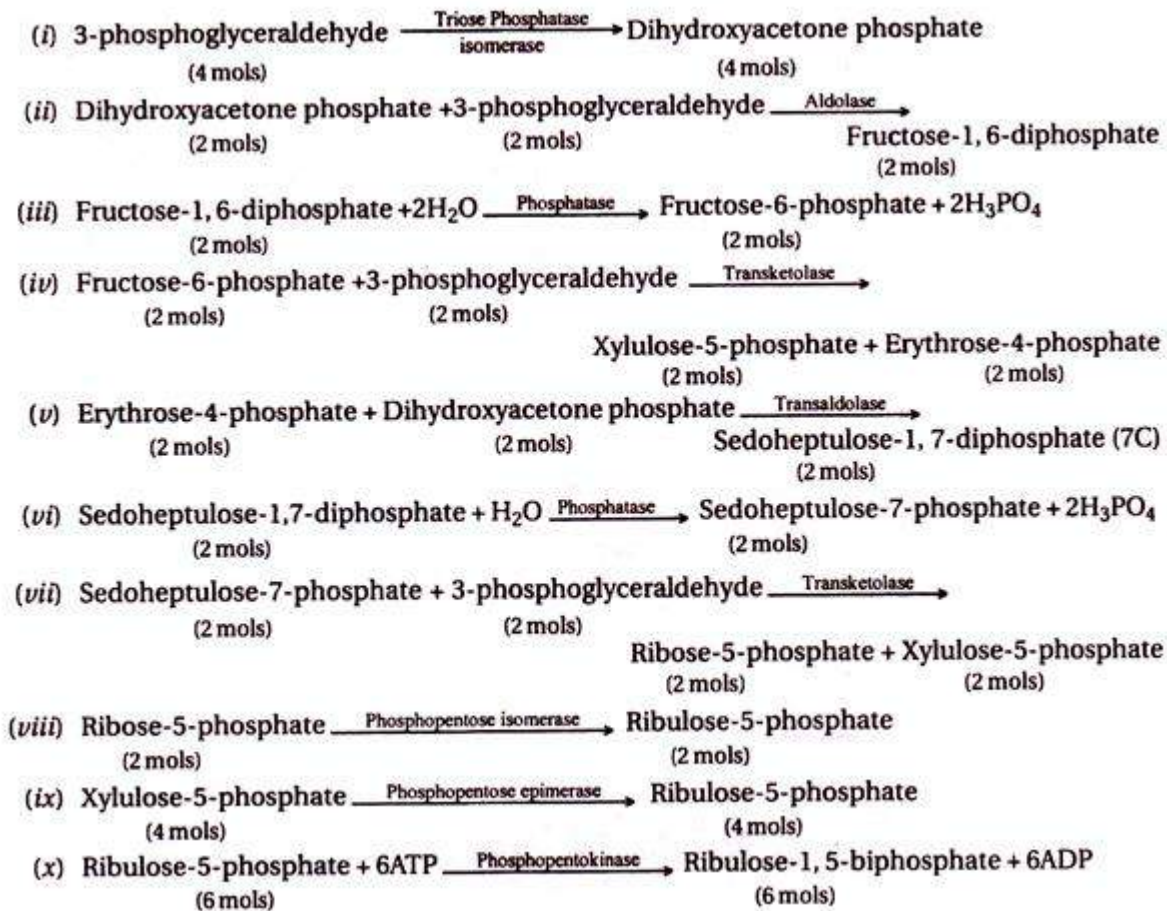
From sucrose are produced other carbohydrate, e.g., starch etc.

5. Regeneration of RUBP:

The remaining PGAL molecules are subjected to several biochemical steps to regenerate 6 molecules of RUBP to complete the cycle.

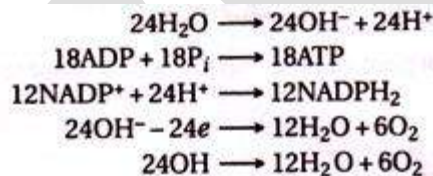
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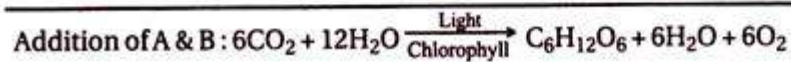
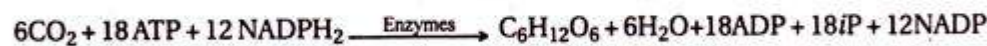
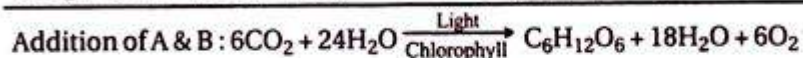
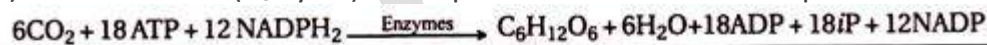


Summary of Photosynthesis:

(A) Light Reaction takes place in thylakoid membrane or granum



(B) Dark Reaction (C_3 cycle) takes place in stroma of chloroplast.



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C₄ Cycle (Hatch and Slack path way OR Kortschak Cycle OR Dicarboxylic acid cycle):

In Graminae members during Dark reaction fixation of carbon di oxide takes place by **C₄ path way**. It is so called because the first intermediate stable compound produced is 4'C' compound oxalo acetic acid. It is composed of 2 carboxyl groups, hence it is also be referred as the **Di-carboxylic acid cycle**. It was first explained by Hatch and Slack hence called **Hatch and Slack Path way**.

This pathway was first reported in members of family Poaceae like sugarcane, maize, sorghum, in subtropical plant like Atriplex spongiosa (Salt bush), Dititaria samguinolis, Cyperus rotundus, Amaranthus etc. and also among certain members of Cyperaceae and certain dicots.

Structural Peculiarities of C₄ Plants (Kranz Anatomy):

- C₄ plants have a characteristic leaf anatomy called **Kranz anatomy**.
- Dimorphic (two morphologically distinct type) chloroplasts occur in C₄ plants.
- The vascular bundles of leaves in C₄ plant are surrounded by a layer of bundle sheath cells that contain large number of chloroplast without grana.
- But mesophyll surrounding this contains small chloroplast with Grana.
- C₄ Path way involves 2 carboxylation reactions. one taking place in chloroplast of Mesophyll and another in chloroplast of Bundle sheath.

In Mesophyll cell:

- (i) Chloroplast is small in size, well developed grannum and less developed stroma.
- (ii) Both PS-II and PS-I are present. Non cyclic photophosphorylation takes place.
- (v) ATP and NADPH₂ produces.
- (vi) Stroma carries PEPCO but absence of RuBisCO.
- (vii) CO₂ acceptor is PEPA (3C) but absence of RUBP
- (viii) First stable product OAA (4C) produces.

In Bundle sheath Cell:

- (i) Size of chloroplast is large
- (ii) Stroma is more developed but granna is poorly developed.
- (iii) Only PS-I present but absence of PS-II
- (iv) Non Cyclic photophosphorylation does not takes place.
- (v) Stroma carries RuBisCO but absence of PEPCO.
- (vi) CO₂ acceptor RUBP (5c) is present but absence of PEPA (3C)
- (vii) C₃-cycle takes place and glucose synthesies.

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(viii) To carry out C₃-cycle both ATP and NADPH₂ comes from mesophyll cell chloroplast.

1. **Fixation of CO₂** Carbon dioxide from atmosphere is accepted by Phosphoenol pyruvic acid (PEPA) present in stroma of mesophyll cell chloroplast and it converts to oxaloacetic acid (OAA) in the presence of enzyme PEPCO (Phosphoenolpyruvate carboxylase).
2. **Formation of Malic acid:** This OAA enters into the chloroplast of bundle sheath cell and there it undergoes oxidative decarboxylation yielding pyruvic acid (3C) and CO₂.
3. The carbon dioxide released in bundle sheath cell reacts with RuBP (Ribulose 1, 5 biphosphate) in presence of RUBISCO and carry out Calvin cycle to synthesize glucose. Pyruvic acid enters mesophyll cells and regenerates PEPA. In C₄ cycle two carboxylation reactions take place.

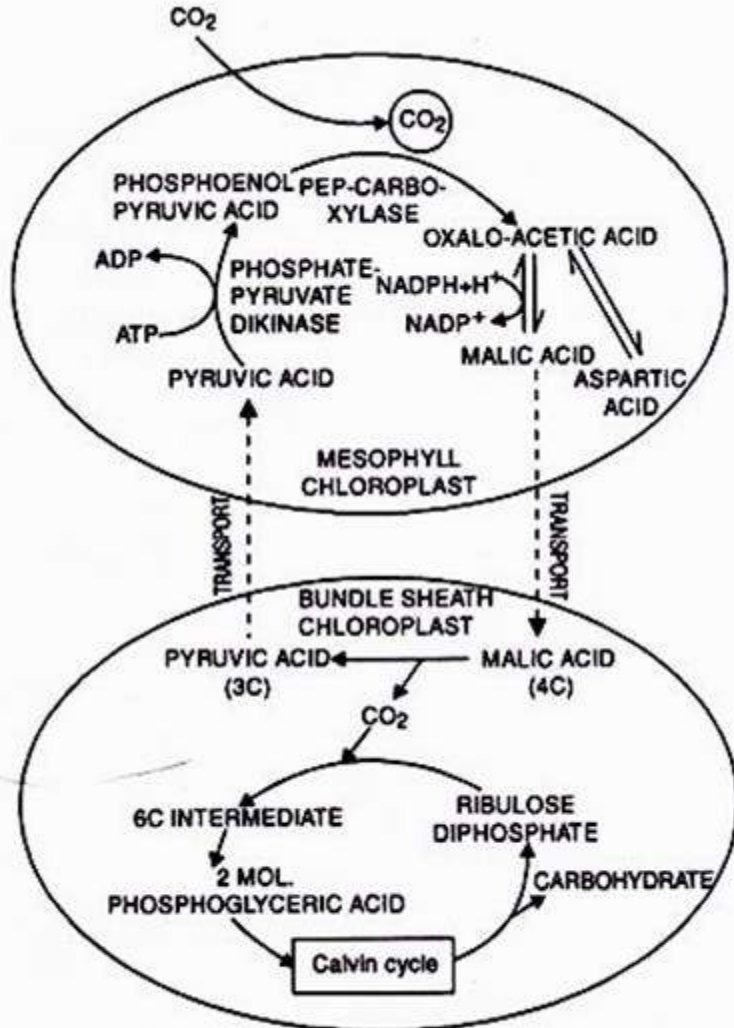


Fig. 6.14. HSK pathway or C₄ cycle

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Reactions taking place in mesophyll cells are stated below: (1st carboxylation)

C₄ plants are better photosynthesizers. There is no photorespiration in these plants. To synthesize one glucose molecule it requires 30 ATP and 12 NADPH₂.

Significance of C₄ Cycle:

1. C₄ plants have greater rate of carbon dioxide assimilation than C₃ plants because PEPCO has great affinity for CO₂ and it shows no photorespiration resulting in higher production of dry matter.
2. C₄ plants are better adapted to environmental stress than C₃ plants.
3. Carbon dioxide fixation by C₄ plants requires more ATP than C₃ plants for conversion of pyruvic acid to PEPA.
4. Carbon dioxide acceptor in C₄ plant is PEPA and key enzyme is PEPCO.
5. They can very well grow in saline soils because of presence of C₄ organic acid.

Crassulacean Acid Metabolism (CAM Pathway):

It is a mechanism of photosynthesis which occurs in succulents and some other plants of dry habitats where the stomata remain closed during the daytime and open only at night. The process of photosynthesis is similar to that of C₄ plants but instead of spatial separation of initial PEPcase fixation and final Rubisco fixation of CO₂, the two steps occur in the same cells (in the stroma of mesophyll chloroplasts) but at different times, night and day, e.g., Sedum, Kalanchoe, Opuntia, Pineapple (Fig. 6.13). (CAM was for the first time studied and reported by Ting (1971).

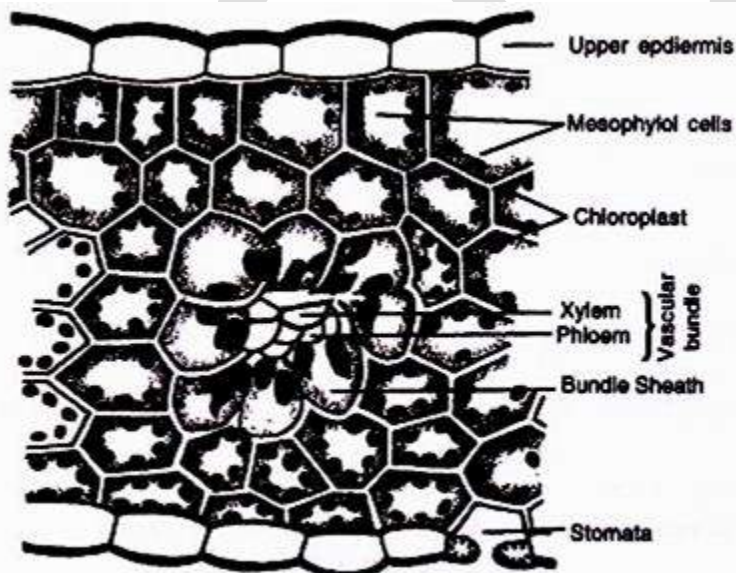


Fig. 6.13. T.S. of monocot plant leaf showing Kranz type of anatomy.

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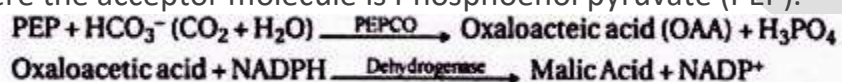
Characteristics of CAM Plants:

1. Stomatal movement is scoto-active.
2. Presence of monomorphic chloroplast.
3. Stroma of chloroplast carries both PEPCO and RUBISCO.
4. Absence of Kranz anatomy.
5. It is more similar to C₄ plants than C₃ plants.
6. In these plants pH decreases during night and increases during day time.

Mechanism of CAM Pathway:

PHASE I. During night:

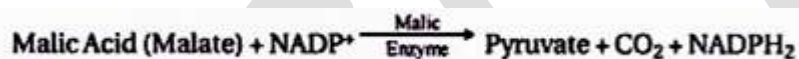
Stomata of Crassulacean plants remain open at night. Carbon dioxide is absorbed from outside. With the help of Phosphoenol pyruvate carboxylase (PEPCO) enzyme the CO₂ is immediately fixed, and here the acceptor molecule is Phosphoenol pyruvate (PEP).



Malic acid is the end product of dark fixation of CO₂. It is stored inside cell vacuole.

PHASE II:

During day time the stomata in Crassulacean plants remain closed to check transpiration, but photosynthesis does take place in the presence of sun light. Malic acid moves out of the cell vacuoles. It is de-carboxylated with the help of malic enzyme. Pyruvate is produced. It is metabolized.



The CO₂ thus released is again fixed through Calvin Cycle with the help of RUBP and RUBISCO. This is a unique feature of these succulent plants where they photosynthesis without wasting much of water. They perform acidification or dark fixation of CO₂ during night and de-acidification during day time to release carbon dioxide for actual photosynthesis.

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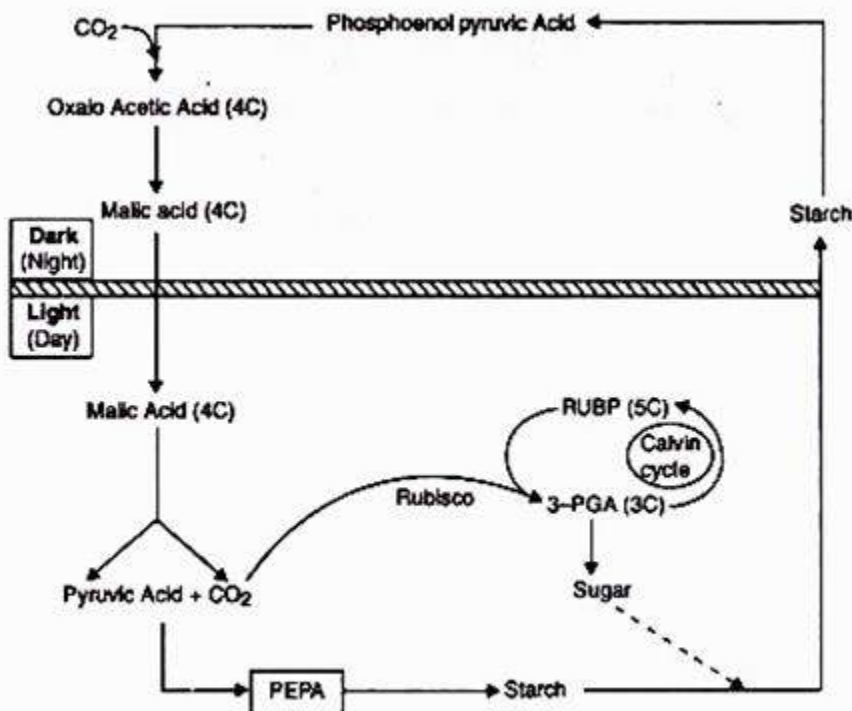


Fig. 6.15. Mechanism of Crassulacean acid metabolism (CAM) showing CO₂ uptake from outside during night and its utilisation in photosynthesis during daytime.

Ecological Significance of CAM Plants:

These plants are ecologically significant because they can reduce rate of transpiration during day time, and are well adapted to dry and hot habitats.

1. The stomata remain closed during the day and open at night when water loss is little due to prevailing low temperature.
2. CAM plants have parenchyma cells, which are large and vacuolated. These vacuoles are used for storing malic and other acids in large amounts.
3. CAM plants increase their water-use efficiency, and secondly through its enzyme PEP carboxylase, they are adapted to extreme hot climates.
4. CAM plants can also obtain a CO₂ compensation point of zero at night and in this way accomplish a steeper gradient for CO₂ uptake compared to C₃ plants.
5. They lack a real photosynthesis during daytime and the growth rate is far lower than in all other plants (with the exception of pineapple).

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Photorespiration or C₂ Cycle or Glycolate Cycle or Photosynthetic Carbon Oxidation Cycle:

Photorespiration is the light dependent process of oxygenation of RUBP (Ribulose bi-phosphate) and release of carbon dioxide by photosynthetic organs of the plant. Otherwise, as we know, photosynthetic organs release oxygen and not CO₂ under normal situation.

Occurrence of photorespiration in a plant can be demonstrated by:

(i) Decrease in the rate of net photosynthesis when oxygen concentration is increased from 2-3 to 21%.

(ii) Sudden increased evolution of CO₂ when an illuminated green plant is transferred to dark.

Photorespiration is initiated under high O₂ and low CO₂ and intense light around the photosynthesizing plant. Photorespiration was discovered by Dicker and Tio (1959), while the term "Photorespiration" was coined by Krotkov (1963). Photorespiration should not be confused with photo-oxidation. While the former is a normal process in some green plants, the latter is an abnormal and injurious process occurring in extremely intense light resulting in destruction of cellular components, cells and tissues.

On the basis of photorespiration, plants can be divided into two groups:

(i) Plants with photorespiration (temperate plants) and plants without photorespiration (tropical plants).

Site of Photorespiration:

Photorespiration involves three cell organelles, viz., chloroplast, peroxisome and mitochondria for its completion. Peroxisome, the actual site of photorespiration, contains enzymes like glycolate oxydase, glutamate glyoxalate aminotransferase, peroxidase and catalase enzymes.

Mechanism of Photorespiration:

We know that the enzyme RUBISCO (Ribulose biphosphate carboxylase oxygenase) catalyzes the carboxylation reaction, where CO₂ combines with RuBP for calvin cycle (dark reaction of photosynthesis) to initiate. But this enzyme RUBISCO, under intense light conditions, has the ability to catalyse the combination of O₂ with RuPB, a process called oxygenation.

In other words the enzyme RUBISCO can catalyse both carboxylation as well as oxygenation reactions in green plants under different conditions of light and O₂/CO₂ ratio. Respiration that is initiated in chloroplasts under light conditions is called photorespiration. This occurs essentially because of the fact that the active site of the enzyme RUBISCO is the same for both carboxylation and oxygenation (Fig. 6.16).

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The oxygenation of RuBP in the presence of O₂ is the first reaction of photorespiration, which leads to the formation of one molecule of phosphoglycolate, a 2 carbon compound and one molecule of phosphoglyceric acid (PGA). While the PGA is used up in the Calvin cycle, the phosphoglycolate is dephosphorylated to form glycolate in the chloroplast (Fig. 6.16).

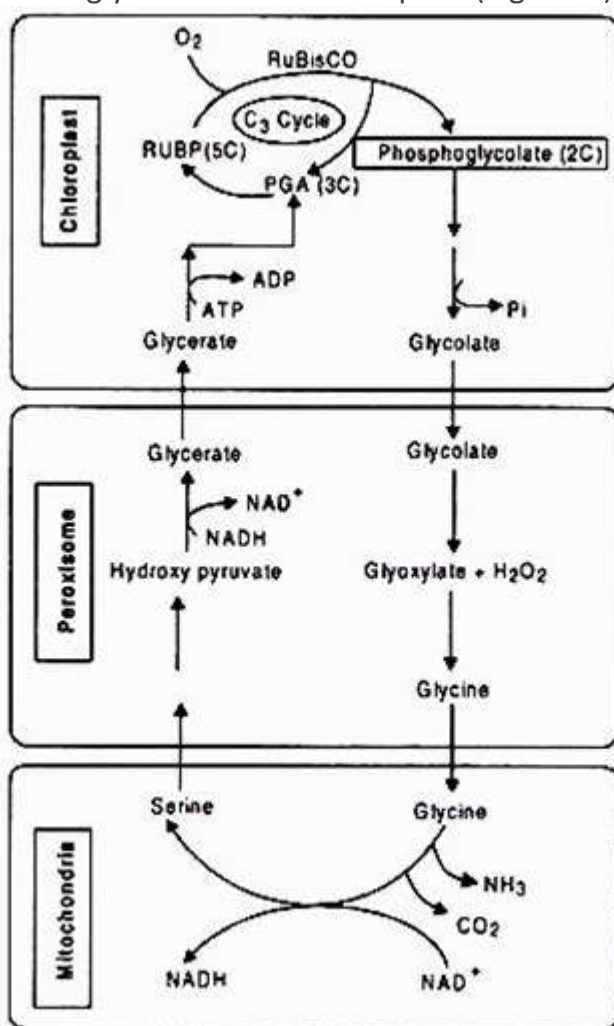
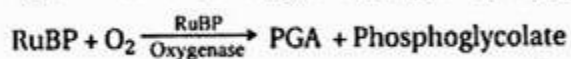


Fig. 6.16. Schematic diagram to explain the mechanism of photorespiratory carbon oxidation cycle in plants.



From the chloroplast, the glycolate is diffused to peroxisome, where it is oxidised to glyoxylate. In the peroxisome, the glyoxylate is used to form the amino acid, glycine. Glycine enters mitochondria where two molecules of glycine (4 carbons) give rise to one molecule of serine (3 carbon) and one CO₂ (one carbon).

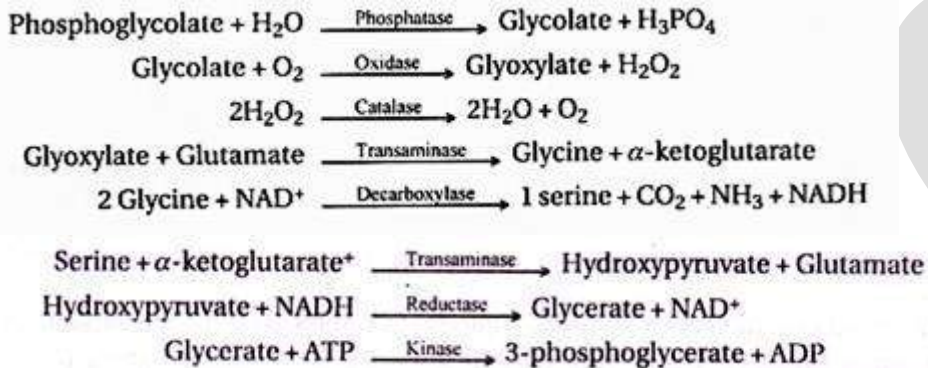
The serine is taken up by the peroxisome, and through a series of reactions, is converted to glycerate. The glycerate leaves the peroxisome and enters the chloroplast, where it is phosphorylated to form

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PGA. The PGA molecule enters the calvin cycle to make carbohydrates, but one CO₂ molecule released in mitochondria during photorespiration has to be re-fixed.

In other words, 75% of the carbon lost by oxygenation of RuBP is recovered, and 25% is lost as release of one molecule of CO₂. Because of the features described above, photorespiration is also called photosynthetic carbon oxidation cycle.



Minimization of Photorespiration (C₄ and CAM Plants):

Since photorespiration requires additional energy from the light reactions of photosynthesis, some plants have mechanisms to reduce uptake of molecular oxygen by Rubisco. They increase the concentration of CO₂ in the leaves so that Rubisco is less likely to produce glycolate through reaction with O₂.

C₄ plants capture carbon dioxide in cells of their mesophyll (using an enzyme called PEP carboxylase), and they release it to the bundle sheath cells (site of carbon dioxide fixation by Rubisco) where oxygen concentration is low.

The enzyme PEP carboxylase is also found in other plants such as cacti and succulents who use a mechanism called Crassulacean acid metabolism or CAM in which PEP carboxylase put aside carbon at night and releases it to the photosynthesizing cells during the day.

This provides a mechanism for reducing high rates of water loss (transpiration) by stomata during the day. This ability to avoid photorespiration makes these plants more hardy than other plants in dry conditions where stomata are closed and oxygen concentration rises.

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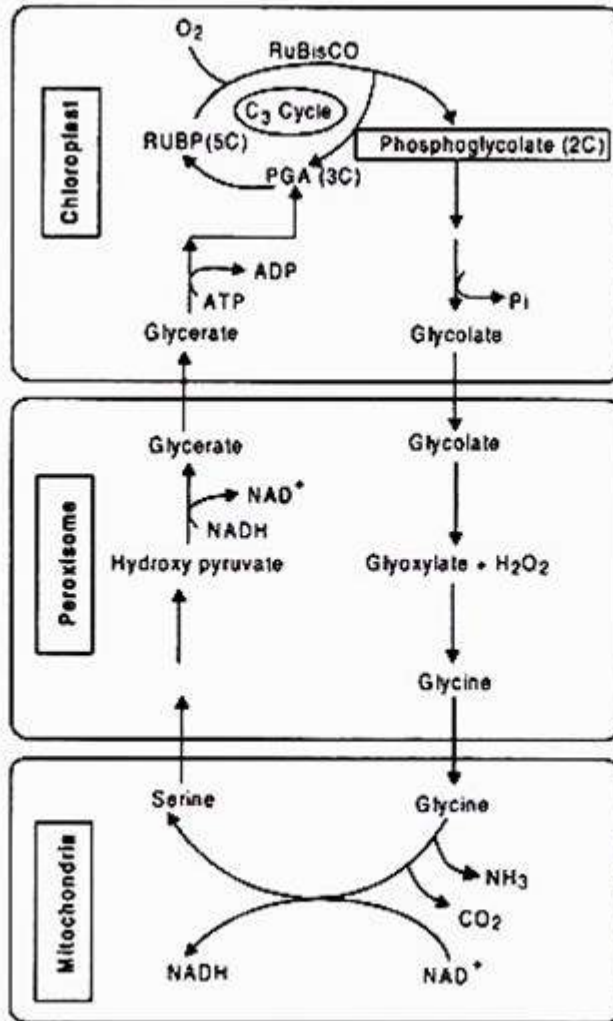


Fig. 6.16. Schematic diagram to explain the mechanism of photorespiratory carbon oxidation cycle in plants.

Factors Affecting Photosynthesis:

Photosynthesis is affected by both environmental and genetic (internal) factors. The environmental factors are light, CO_2 , temperature, soil, water, nutrients etc. Internal or genetic factors are all related with leaf and include protoplasmic factors, chlorophyll contents, structure of leaf, accumulation of end product etc.

Principle of Limiting Factors:

Liebig (1843) proposed law of minimum which states that the **rate of a process is limited by the pace (rapidity) of the slowest factor**. It was later on modified by Blackman (1905) as "**principle of limiting factors**". It states that **when a metabolic process is conditioned as to its rapidity by a number of separate factors, the rate of the process is limited by the pace (rapidity) of the slowest factor**. This principle is also known as "**Blackman's Law of Limiting Factors.**"

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A metabolic process is conditioned by a number of factors. The slowest factor or the limiting factor is the one whose increase in magnitude is directly responsible for an increase in the rate of the metabolic process (here photosynthesis).

At a given time, only the factor that is most limiting among all will determine the rate of photosynthesis. For example, if CO₂ is available in plenty but light is limiting due to cloudy weather, the rate of photosynthesis under such a situation will be controlled by the light. Furthermore, if both CO₂ and light are limiting, then the factor which is the more limiting of the two, will control the rate of photosynthesis.

- Blackman (1905) studied **the effect of CO₂ concentration, light intensity and temperature on rate of photosynthesis**. All other factors were maintained in optimum concentration.
- Initially the photosynthetic material was kept at 20°C in an environment having 0.01% CO₂. When no light was provided to photosynthetic material, it did not perform photosynthesis. Instead, it evolved CO₂ and absorbed O₂ from its environment. He provided light of low intensity (say 150 foot candles) and found photosynthesis to occur.
- When light intensity was increased (say 800 foot candles), the rate of photosynthesis increased initially but soon it levelled off.
- The rate of photosynthesis could be further enhanced only on the increase in availability of CO₂. Thus, initially light intensity was limiting the rate of photosynthesis.
- When sufficient light became available, CO₂ became limiting factor. When both are provided in sufficient quantity, the rate of photosynthesis rose initially but again reached a peak. It could not be increased further.
- It was found that increase in temperature could raise the rate of photosynthesis up to 35°C. Further increase was not possible. At this stage, some other factor became limiting. Therefore, at one time only one factor limits the rate of physiological process.

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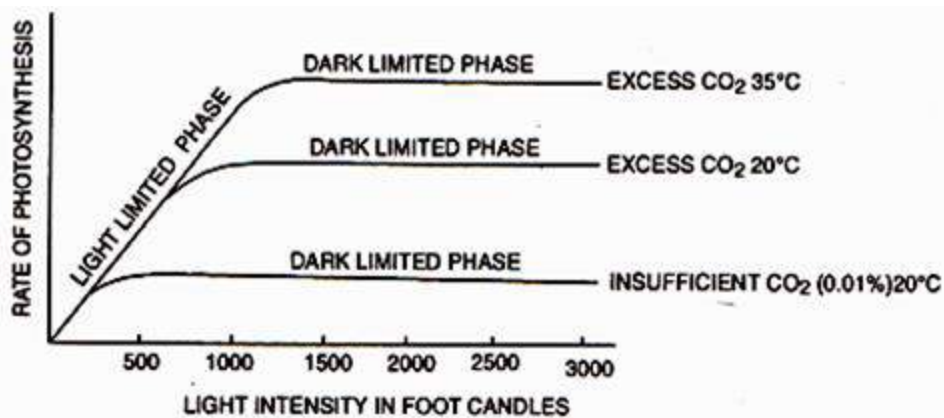


Fig. 6.17. Blackmann's law of limiting factors.

Objections of Blackman's law of limiting factors.

- (i) It has been observed that the rate of a process cannot be increased indefinitely by increasing the availability of all the known factors;
- (ii) The principle of Blackman is not operative for toxic chemicals or inhibitors and
- (iii) The pace of reaction can be controlled simultaneously by two or more factors.

Unit 4: Respiration(6 Hrs)

Structure of mitochondrion, Glycolysis, anaerobic respiration, TCA cycle; Oxidative Phosphorylation, Pentose Phosphate Pathway.

INTRODUCTION:

Carbohydrate molecules synthesised during photosynthesis is stored as glucose and starch. Organisms use such energy for their activities by oxidising them into simple low energy molecules, i.e., carbon dioxide and water. The reactions involved in **process of oxidation** are known as respiration. The compounds that are oxidised during process of respiration are called **respiratory substrates**.

Definition:-

Respiration is defined as a process of breakdown of complex high energy food molecules into simple low energy molecules like CO₂ and H₂O, releasing the energy in living cells.

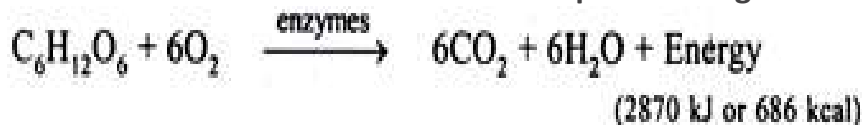
- The energy released during oxidation of energy rich compounds is made available for activities of cells through an intermediate compound called **adenosine triphosphate (ATP)**.
- The whole of energy contained in respiratory substrates is released slowly in several steps of reactions controlled by different enzymes.

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- Respiration takes place in all types of living cells, called **cellular respiration**.
- During respiration oxygen is utilised, and CO₂ water and energy are released as products.
- The released energy is utilised in various energy-requiring activities of the organisms, and the carbon dioxide released during respiration is used for biosynthesis of other molecules in the cell.

The reaction that occurs in common respiration of glucose may be summed up as follows:



Here, 686 kcal or 2870 kJ of energy is liberated per molecule of glucose. Formerly, this calculated value was 673 kcal. One kcal is equal to 1000 calories. This means that one molecule of glucose on complete oxidation yields 686 kcal (kilocalories) of energy, (i.e., 686, 000 calories).

The main facts associated with respiration are:

- a. Consumption of atmospheric oxygen.
- b. Oxidation and decomposition of a portion of the stored food resulting in a loss of dry weight as seen in the seeds germinating in dark.
- c. Liberation of carbon dioxide and a small quantity of water (the volume of CO₂ liberated is equal to volume of O₂ consumed).
- d. Release of energy by breakdown of organic food, (such as carbohydrates).

Respiratory Substrates:

Respiratory substrates are organic substances which are oxidised during respiration. They may be carbohydrates, fats and proteins.

Types of Respiration:

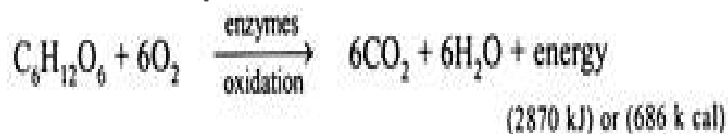
There are two main types of respiration:

- (i) Aerobic, and (ii) Anaerobic.

(i) Aerobic Respiration:

Aerobic respiration leads to a **complete oxidation** of stored food (organic substances) in the presence of oxygen, and releases carbon dioxide, water and a large amount of energy Ex: higher organisms.

The overall equation is:



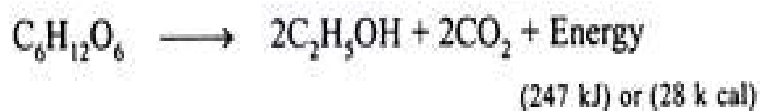
(ii) Anaerobic respiration:

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Anaerobic respiration occurs in complete absence of oxygen. Incomplete oxidation of stored food and formation of carbon dioxide and ethyl alcohol, and sometimes also various organic acids, such as malic, citric, oxalic, tartaric, etc. Very little energy is released by this process to maintain activity of protoplasm.

EX: bacteria and fungi. Many tissues of higher plants, seeds in storage, fleshy fruits, and succulent plants, such as cacti temporarily take anaerobic respiration. **The equation is as follows:**



This process of oxidation in microbes is known as fermentation. This is quite similar to that of anaerobic respiration in case of higher plants.

Mechanism of Respiration:

There are two major phases of respiration:

- (i) Glycolysis, and
- (ii) Krebs cycle.

- During respiration, carbohydrates are converted into pyruvic acid through a series of enzymatic reactions. This series of reactions is known as **glycolysis** which takes place in **cytosol**.
- Pyruvic acid enters mitochondria, where several enzymes catalyse the reactions, and pyruvic acid finally converts into CO₂ and water. This series of enzymatic reactions is known as Krebs cycle.

Glycolysis(Greek words, glycos = sugar and lysis = splitting).

The scheme of glycolysis was discovered by three German Scientists, Gustav **Embden**, Otto **Meyerhof** and J. **Parnas**, and therefore, referred as **EMP pathway**. Glycolysis is common to both aerobic and anaerobic respiration.

In anaerobic organisms, this is only process in respiration. Glycolysis occurs in cytoplasm of cells. During this process, glucose undergoes partial oxidation to form two molecules of pyruvic acid.

The main steps of glycolytic pathway are as follows:

a. Phosphorylation of Sugar (i.e., First Phosphorylation):

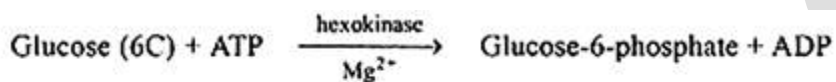
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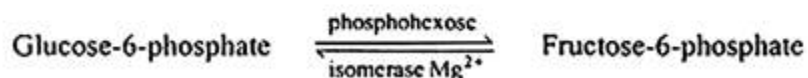
Glucose and fructose are phosphorylated to give rise to **glucose-6-phosphate and fructose-6-phosphate**, respectively, by the activity of enzyme **hexokinase**, in presence of ATP. The phosphorylated form of glucose then isomerises to produce fructose-6-phosphate. Isomerisation takes place with the help of enzyme phosphohexose isomerase.

Further steps of metabolism of glucose and fructose are quite similar.

Equations are as follows:

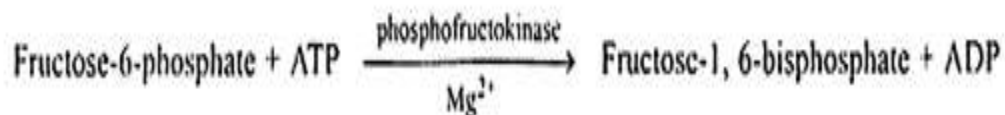


Now isomerisation occurs :



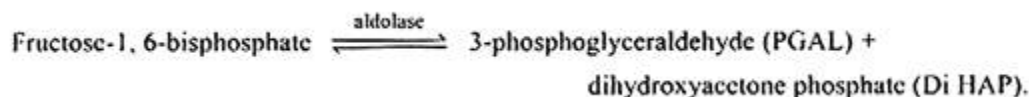
b. Phosphorylation of Fructose-6-Phosphate (i.e., Second Phosphorylation):

Fructose-6-phosphate is phosphorylated and **fructose-1, 6-bisphosphate** produced by the action of enzyme **phosphofructokinase** in presence of ATP.



c. Splitting:

Fructose- 1, 6-bisphosphate splits into two molecules of **triose phosphate, i.e., 3-phosphoglyceraldehyde (PGAL) and dihydroxyacetone phosphate (Di HAP)**, which are interconvertible.

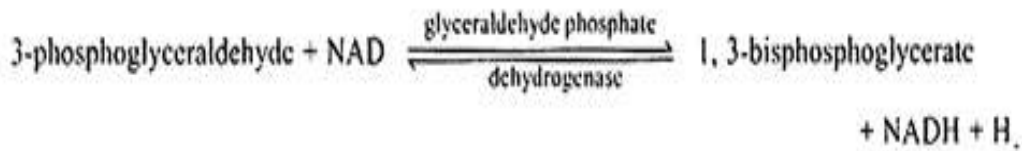


d. Oxidative Dehydrogenation:

After formation of 3-phosphoglycerldehyde (PGAL) is oxidized to a carboxylic acid, i.e., **1, 3-bisphosphoglycerate**, and NAD is reduced to NADH.

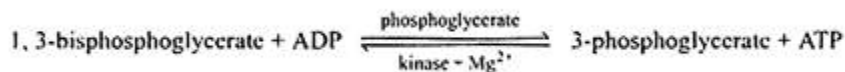
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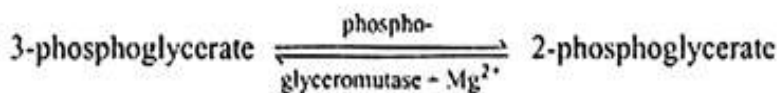
e. Formation of ATP:

1, 3-bisphosphoglycerate is converted into **3-phosphoglycerate** by enzyme **phosphoglycerate kinase**, and ATP is generated during this process. Direct synthesis of ATP from intermediate metabolites is called substrate level phosphorylation.



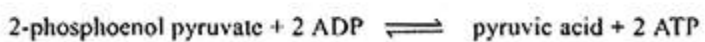
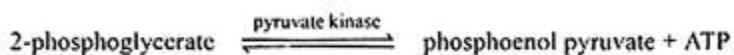
f. Isomerisation:

3-phosphoglycerate is converted into its isomer **2- Phosphoglycerate** by catalytic activity of enzyme **phosphoglyceromutase**.



g. Dehydration:

2-phosphoglycerate converts into **phosphoenol pyruvate** (PEP) in the presence of enzyme **pyruvate kinase** and liberates ATP.



Generation and Utilisation of ATP during Glycolysis:

During glycolytic pathway, the molecules of ATP are produced by

(i) Direct transfer of phosphate to ADP and (ii) Oxidation of NADH produced during glycolytic pathway to NAD⁺.

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i. In the end of glycolysis net gain of ATP:

(i) During glycolysis two triose phosphate molecules are formed from one glucose molecule, and **4 ATP molecules are produced**.

(ii) Out of 4 ATP molecules, 2 ATP molecules are utilised in first few steps in converting glucose to fructose-1, 6 biphosphate.

(iii) **3 ATP molecules are produced** from oxidation of each of two molecules of NADH produced during catabolism of glucose.

(iv) In all, a **net gain of 8 molecules** occurs during process of glycolysis.

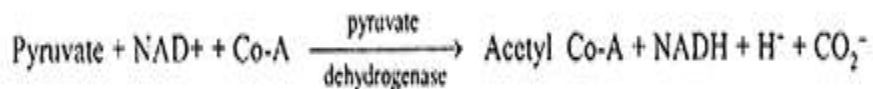
(v) In anaerobic respiration, $\text{NADH} + \text{H}^+$ is not converted to ATP, and therefore, only 2 ATP molecules are produced.

Oxidative Decarboxylation Pyruvic Acid :(Aerobic Oxidation of Pyruvic Acid)

Pyruvic acid generated in cytoplasm through glycolysis is transferred to mitochondria. One of the three carbon atoms of pyruvic acid is oxidised to carbon dioxide in a reaction called “**oxidative decarboxylation**”.

Pyruvate is decarboxylated, and later oxidised by enzyme pyruvate dehydrogenase. This enzyme is made up of a decarboxylase, lipoic acid, TPP, transacetylase and Mg^{+2} . Acetyl Co-A acts as substrate entrant for Krebs cycle.

The equation is as follows:



Acetyl Co-A can enter into mitochondria while pyruvic acid cannot.

Acetyl Co-A can enter into mitochondria while pyruvate acid cannot.

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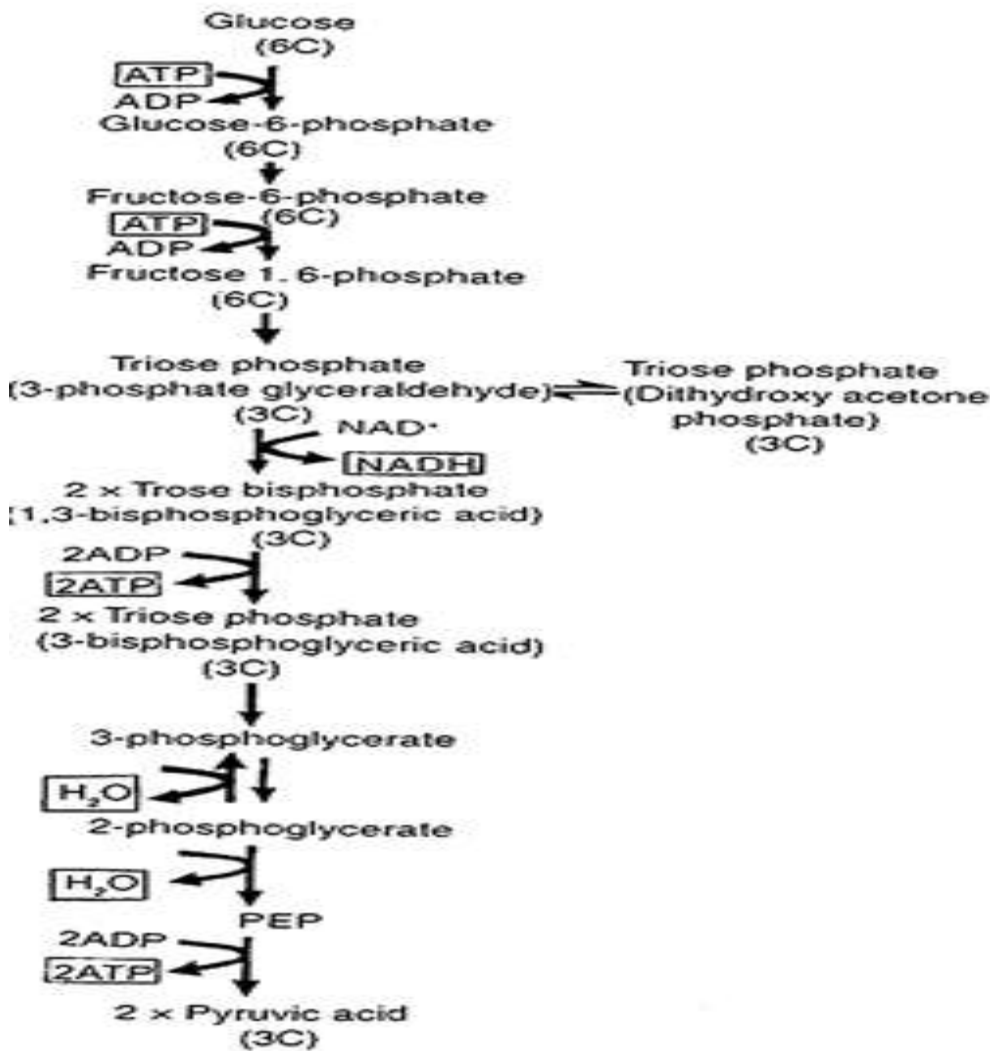


Fig. 4.2. Main steps of glycolysis or EMP pathway.

Krebs Cycle:

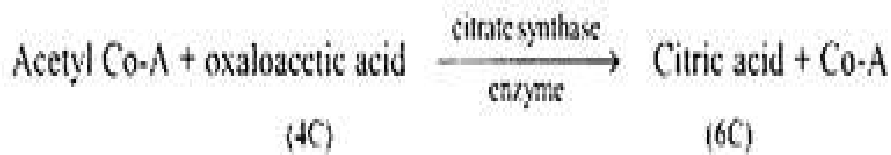
- Sir Hans Adolf Krebs, discovered role of pyruvate in conversion of glucose hydrogens into fumarate.
- He discovered, in 1937, tricarboxylic acid cycle (i.e., TCA cycle), also known as Citric acid cycle or Krebs cycle.
- Citric acid cycle occurs in matrix of mitochondria.
- In Krebs cycle first **acetyl Co-A** enter into a reaction to form citric acid. Krebs explained explained how pyruvate is broken down to CO₂ and H₂O. For this pioneer work Krebs was awarded Nobel Prize in 1953.

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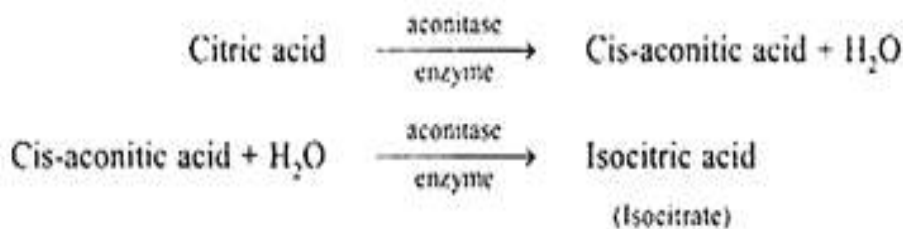
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Various steps of Krebs are as follows:

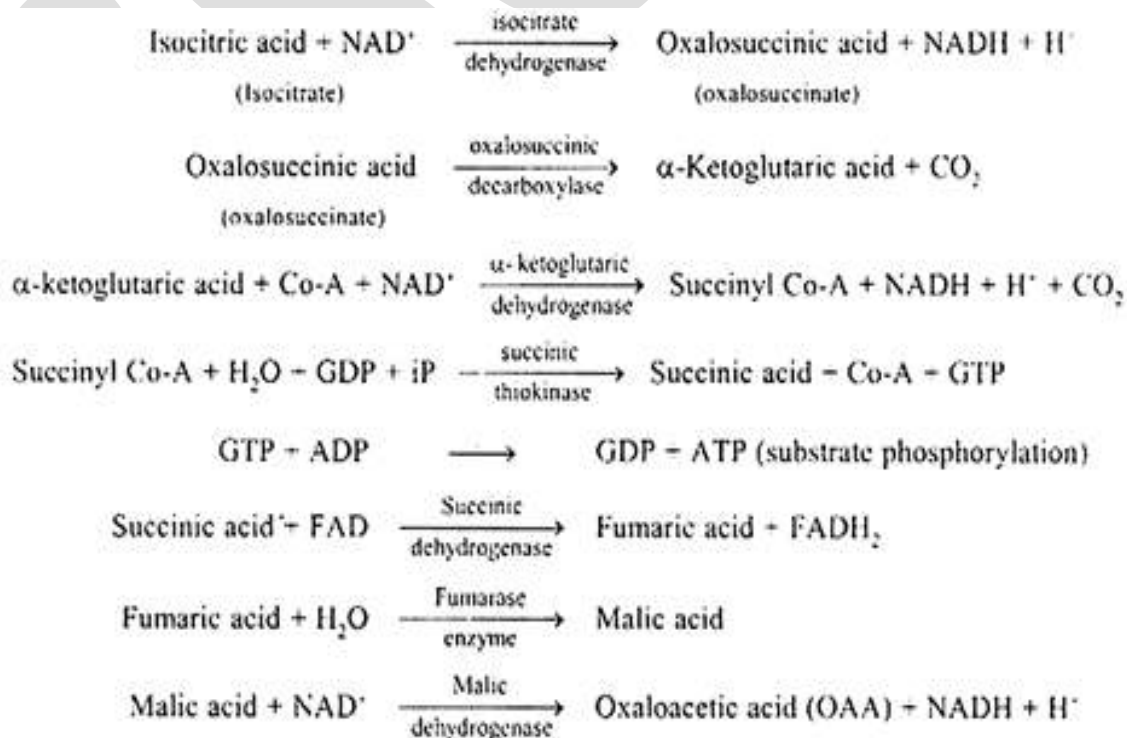
1. This is the first reaction of Krebs cycle, where one molecule of acetyl Co-A combines with 4-carbon oxaloacetic acid (OAA); in presence of Citrate synthase to form 6-carbon citric acid is produced, and Co-A is released.



2. Citrate (citric acid) is isomerised to isocitrate (isocitric acid).



3. Cis-aconitic acid is converted into isocitric acid with the addition of water in the presence of iron containing enzyme aconitase.



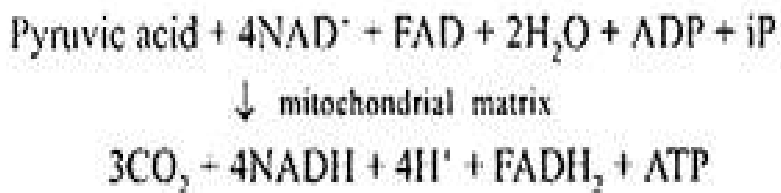
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4. During citric acid cycle (Krebs cycle) 3 molecules of NAD^+ and one molecule of FAD (Flavin Adenine Dinucleotide) are reduced to produce NADH and FADH_2 , respectively.

During citric acid cycle NADH and FADH_2 are produced. Now, they are linked with electron transport system (ETS) and produce ATP by oxidative phosphorylation.

This may be summarised in following equation:



In the end of Krebs cycle, glucose molecule is completely oxidised. **From one glucose molecule, two pyruvic acid molecules are formed.** After oxidation of one pyruvic acid molecule, three CO_2 molecules are released. Thus, in all 6 molecules of CO_2 are released.

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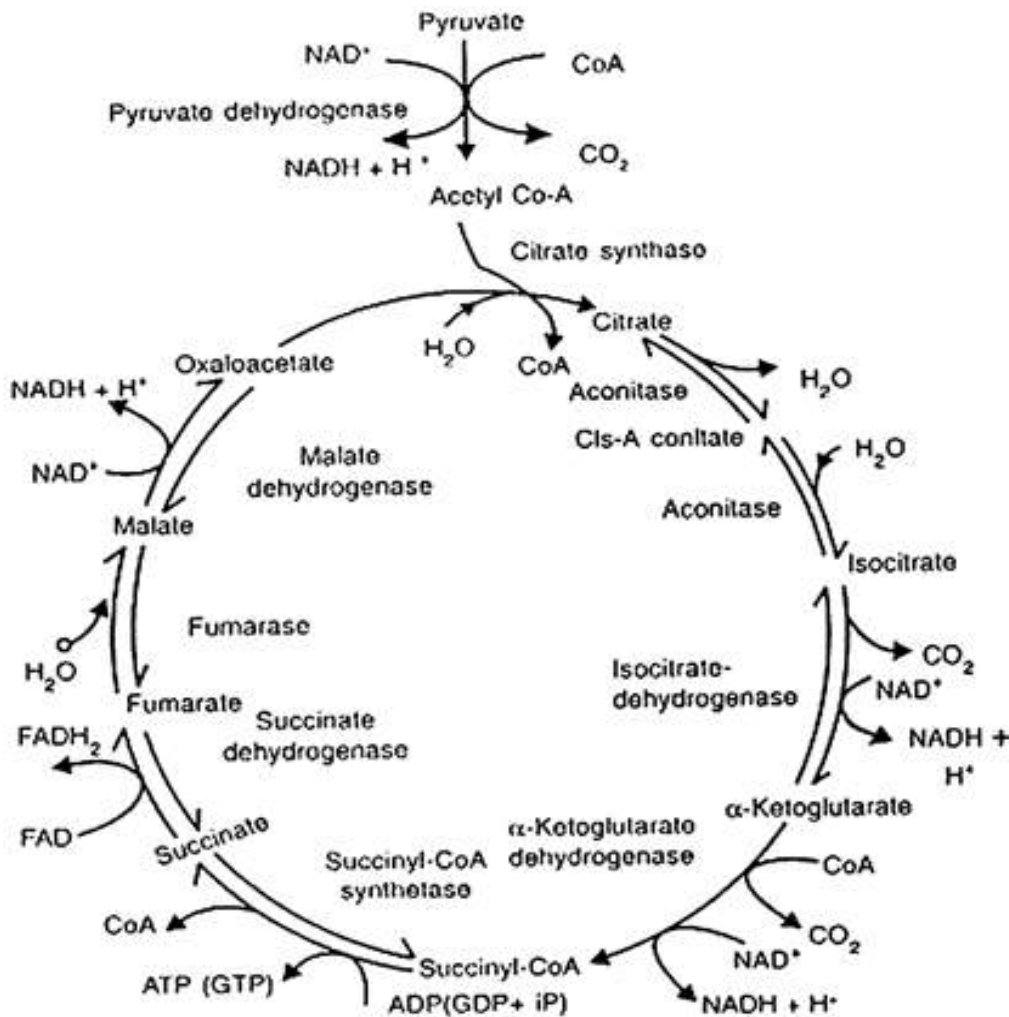


Fig. 4.3. Krebs cycle or citric acid cycle.

Electron Transport System (ETS):

By the end of Krebs cycle, glucose molecule oxidises completely, but the energy does not release till NADH and FADH₂ oxidise through electron transport system (ETS).

The metabolic pathway through which electron passes from one carrier to another, is called electron transport system (ETS). It is also known as electron transport chain or mitochondrial respiratory chain.

- Electron transport system is operative in the inner mitochondrial membrane.
- The electron transport system consists of a series of coenzymes and cytochromes that take part in passage of electrons from a chemical to its ultimate acceptor with a loss of energy at each step.

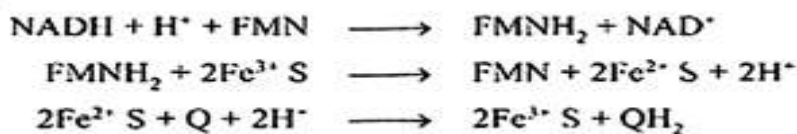
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- The electron carriers include flavins, iron sulphur complexes, quinones and cytochromes. Most of them are prosthetic groups of proteins.
- Electron transport system in mitochondria consists of four complexes which are found in bases of stalked particles in the inner mitochondrial membrane, and also ubiquinone (UQ) or coenzyme Q and cytochrome c which are not bound to stalked particles but act as mobile electron carriers between the complexes.

1. Complex-I:

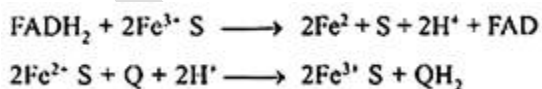
It consists of NADH-dehydrogenase or NADH-Q reductase which contains a flavoprotein FMN (flavin mononucleotide) and is associated with iron-sulphur (Fe-S) proteins. This complex is responsible for passing electrons (also protons) from mitochondrial NADH to ubiquinone (UQ), located within inner mitochondrial membrane.



2. Complex-II:

It consists of succinate dehydrogenase which contains a flavoprotein FAD (flavin adenine dinucleotide) in its prosthetic group and is associated with non heme iron-sulphur (Fe S) proteins.

This complex receives electrons (also protons) from succinic acid and passes them to ubiquinone (UQ). Ubiquinone also receives reducing equivalents via FADH_2 that is generated during oxidation of succinate.



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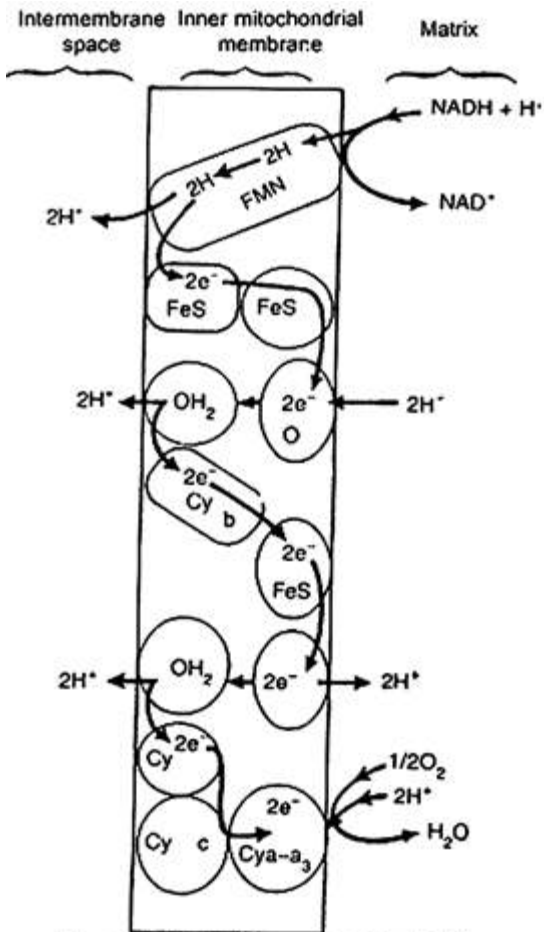


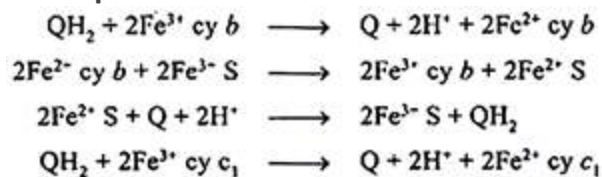
Fig. 4.4. Electron transport system (ETS)

3. Complex-III:

It consists of ubiquinol, cytochrome c and cytochrome bc_1 . The reduced ubiquinone is called ubiquinol. Here ubiquinol is oxidised with the transfer of electrons to cytochrome c via cytochrome bc_1 . It acts as a mobile carrier for transfer of electrons between complex III and complex IV.

This complex is called QH_2 -cytochrome c reductase complex. This bears three components, i.e., cytochrome b, non-heme iron sulphur (Fe - S), and cytochrome c_1 . Coenzyme Q is also involved between Fe-S and cytochrome c_1 .

The equations are as follows:



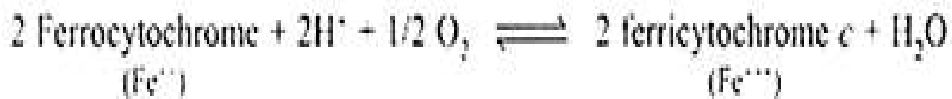
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Now, cytochrome c, transfers electrons to cy c. Like coenzyme Q, cy c is also mobile carrier of electrons.

4. Complex-IV:

It is known as cytochrome c oxidase complex. This contains cytochromes a and a₃, along with two copper centres. This complex receives electrons from cytochrome c and passes them to 1/2 O₂. Two protons are needed and H₂O molecule is formed (terminal oxidation). Here, O₂ is ultimate acceptor of electrons. It combines with protons to form metabolic water or respiratory water.



5. Complex-V:

When electrons are transferred from one carrier to next carrier via complexes 1 to IV in electron transport system (ETS), they are coupled to ATP synthase enzyme complex for production of ATP from ADP and inorganic phosphate (iP).

Number of ATP molecules synthesised during ETS. Depends on nature of electron donor.

- **Oxidation of one molecule of NADH gives rise to 3 molecules of ATP.**
- One molecule of FADH₂ gives rise to 2 molecules of ATP. ATP synthase complex is called complex V.
- During transportation of electrons, hydrogen atoms split into protons and electrons. The electrons are carried by cytochromes.
- Hydrogen atom is accepted by oxygen to form water; the electrons again recombine with their protons.

Oxidative Phosphorylation:

- The process of production of ATP by phosphorylation of ADP in presence of oxygen and ATP synthase is called **oxidative phosphorylation**.
- ATP synthase, also known as complex V. It consists of two major components, i.e., F₁, and F₀.

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- The F_1 headpiece is a peripheral membrane protein complex and contains the site for ATP from ADP and inorganic phosphate (iP).
- F_0 is an integral membrane mitochondrial-protein complex which forms the channel through which protons cross the inner membrane.
- The passage of protons through the channel is coupled to the catalytic site of the F_1 component for the production of ATP.
- Oxidation of one molecule of $NADH_2$ produces 3 ATP molecules whereas a similar oxidation of $FADH_2$ produces 2 ATP molecules

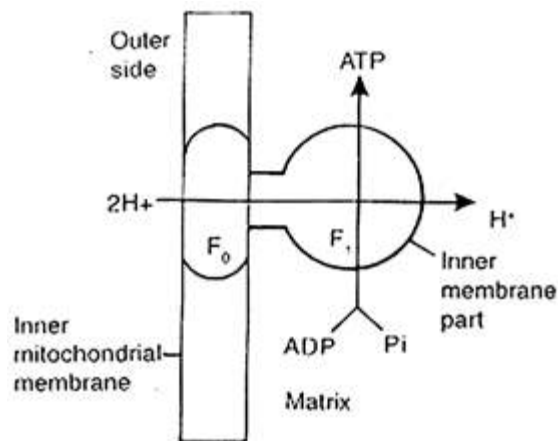


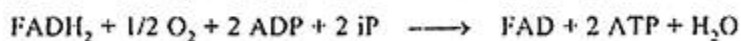
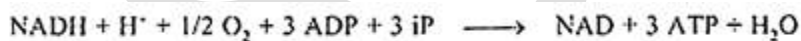
Fig. 4.5. Synthesis of ATP by inner membrane particles of mitochondrion.

Net gain of ATP:

Complete oxidation of glucose to CO_2 and water shows that there is a net gain of 38 ATP.

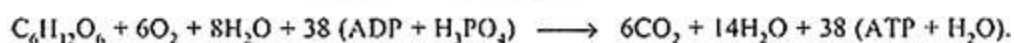
Each $NADH + H^+$ produces 3 ATP molecules,

$FADH_2$ forms only 2 ATP molecules at the end of reaction.



Thus, total gain of ATP in aerobic respiration is as follows:

Glycolysis	→	8 ATP
Pyruvic acid	→	Acetyl Co-A → 6 ATP
Krebs cycle	→	24 ATP
Total	→	38 ATP



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In eukaryotic cells, 2 molecules of ATP are required for transport of NADH produced in glycolysis into mitochondrion for further oxidation, therefore, **net gain of ATP is 36 molecules.**

Significance of Krebs cycle:

- a. During Krebs cycle, carbon skeletons are obtained for use in growth and maintenance of the cell.
- b. Many intermediate compounds are formed which are used in synthesis of other biomolecules, such as amino acids, nucleotides, chlorophyll, cytochromes and fats.
- c. Krebs cycle is major pathway for generation of ATP molecules, which make energy currency of the cell.
- f. Energy is released from glucose, and is used in various biochemical reactions.

Unit 5:Enzymes 5 Hrs

Structure, Nomenclature, Properties, classification; Mechanism of enzyme action and enzyme inhibition.

Unit 6:Nitrogen metabolism 4 Hrs

Biological nitrogen fixation; Nitrate Metabolism, Synthesis of amino acids, Reductive and Transamination.

NITROGEN METABOLISM

Nitrogen is an important Macro element required by plants for their nitrogen metabolism. It is the main source of compounds like Ammonia, Proteins , Nucleic acids ,Chlorophyll etc.

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The process of conversion of Molecular Nitrogen of the atmosphere into Nitrogenous compounds of the soil to make it available for absorption by plants is called 'Nitrogen fixation'. It can be grouped into 2 types . Namely

1. Physical nitrogen fixation.
2. Biological Nitrogen fixation.

1. PHYSICAL NITROGEN FIXATION

Conversion of Atmospheric nitrogen into usable form by lightning and rainfall is called Physical nitrogen fixation.

During thunder and lightning gaseous nitrogen is oxidized to nitrogen peroxide which combines with rain water to form nitrous and nitric acid . In the soil with calcium and Potassium form calcium nitrate and Potassium nitrate which are absorbed by plants.

2 .BIOLOGICAL NITROGEN FIXATION

The Process of conversion of atmospheric nitrogen into usable form by soil micro organisms is called Biological nitrogen fixation.It takes place by 3 methods as follows:-

1. **Symbiotic Nitrogen fixation**
2. **Non-symbiotic Nitrogen fixation.**
3. **Associative Nitrogen fixation.**

I Symbiotic Nitrogen fixation

The process of conversion of atmospheric nitrogen to usable forms by Micro organisms when they are present in symbiotic association is called Symbiotic Nitrogen fixation.
Ex : Nitrogen fixation by bacteria Rhizobium when present in Leguminous root nodules.

Mechanism of Nodule formation

Nodules are small ,knob like protruberances formed by species of Rhizobium . Beijerinck isolated it first from Roots of leguminous plants . Prasmukifirst studied mechanism of nodule formation . It involves following steps:-

1. Specific Leguminous root stimulate specific Rhizobium to develop symbiotic association.EX: Pea stimulates Rhizobium leguminosarum , Bean roots stimulates Rhizobium phaseoli etc .

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2. Rhizobium secrete growth hormone IAA which induce curling of root hair that help in attachment of Rhizobium to binding site of Root hair.
3. Rhizobium secretes Cystase enzyme which help in entry of Rhizobium into Root hair.
4. Root hair develops infection thread . It carries the bacteria ,grow across cortical cell , reaches innermost cortical cell releases rhizobium into il.
5. Rhizobial cell induce cortical cell to proliferate. Thus root nodules are formed.

Rhizobium divide ,loose cell wall,become vacuolated ,bulged ,branched cells called bacterioids. These fix nitrogen as plant matures.

Mechanism of symbiotic Nitrogen fixation.

symbiotic Nitrogen fixation Rhizobium in root nodules of leguminous plants requires following factors:-

Symbiotic Nitrgen fixation by Rhizobium in root nodules of leguminous plants requires following factors :-

1. **6 electrons** :- Ferredoxin of Photosynthetic electron transport chain donates electrons.
2. **6 Hydrogen atoms +12ATP** :- Pyruvic acid metabolism donates Hydrogen atoms and ATP molecules ,Respiratory metabolites also supply ATP .
3. **Nitrogenase enzyme** :- '**NIF**' gene of Rhizobium codes for it..



The process of symbiotic nitrogen fixation takes place as follows :-

1. Reduced Ferredoxin donates electrons to oxidized Fe- protein complex ,then this complex become reduced.
2. Reuduced Fe protein complex reacts with ATP and become ATP activated Fe- protein complex . It donates electrons to MO-Fe protein complex.
3. Mo-Fe Protein complex receives electrons from ATP activated Fe- protein Complex and reduced. It transfer electrons to nitrogen.
4. Nitrogen combines with hydrogen to form free Ammonia . It get converted into Amino acids in the root nodules . Amino acids diffuses into soil and become available for the plants.

In Rhizobium Nitrogenase enzyme needs anaerobic condition to fix molecular Nitrogen .Leg haemoglobin in the root nodule absorbs oxygen and keeps Rhizobium in oxygen free state.

[Leg haemoglobin is a protein produced by symbiotic interaction of rhizobium and Legume Root]. Haeme component of protein is formed by Rhizobium globulin by legume root].

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Amino acid synthesis

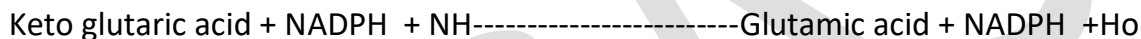
Amino acids are building blocks of proteins. carboxyl group amino acids synthesis takes place in 2 steps. They are :-

- 1.Reductive amination
2. Trans amination

1 .Reductive amination : The process of conversion of inorganic Nitrogen into organic Nitrogen by Amination and Reduction at alpha keto group of organic acid is called **Reductive amination.**

Inorganic Nitrogen, in the form of nitrogen Ammonia is produced by Biological nitrogen fixation or obtained from the soil or by reduction of nitrogen.

Inorganic nitrogen reacts with alpha keto glutaric acid in presence of an enzyme glutamic dehydrogenase and NADPH to form Amino acid.



2 Transamination:The transfer of an amino group from Glutamic acid to keto position of corresponding keto acid in presence of an enzyme transaminase and co enzyme pyridoxyl phosphate is called **“Transamination”**.

The co enzyme Pyridoxyl phosphate acts as a carrier of amino group . It picks amino group from donor amino acid and converts into pyridoxyl amine phosphate. It transfers this amino group to the acceptor keto acid forming new amino acid and itself is converted into pyridoxyl phosphate.

II Non symbiotic Nitrogen fixation

The process of conversion of molecular Nitrogen of the atmosphere into free Ammonium ions by free living bacteria is known as **Non symbiotic nitrogen fixation.** EX; Azatobacter, clostridium, Pseudomonas, Chlorobium , Rhodospirillum etc .

III Associative Nitrogen fixation

The process of conversion of molecular nitrogen of the atmosphere into free Ammonium ions by free living bacteria when present in intimate association with roots of higher plants is known as **“ Associative Nitrogen fixation.”**

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It is an association between bacteria and roots of cereals , grasses without development of nodule like structure. In this type bacteria live in Rhizosphere [transition zone between soil & root] .the bacteria fix Nitrogen and supply to root, in return roots provide carbon di oxide , & carbohydrates to bacteria.

Ex: Beijerinckia ----Roots of Sugar cane ,

Azospirillum -----Roots of cereals

Azotobacter paspali-----Roots of tropical grass.

NITROGEN CYCLE

The cyclic movement of Nitrogen between organic and inorganic form to maintain its balance in nature is called” Nitrogen cycle”.

Nitrogen cycle involves following steps :-

1. Air is a reservoir for nitrogen. It is made available for plants during lightning and rain fall by physical means and through soil microbes by biological nitrogen fixation.
2. Nitrates are utilized by green plants to synthesise nutrients .[proteins].
3. Animals feed on plants , Nutrients are passed to animals . These excrete nitrogenous wastes. These are converted into Nitrites by Ammonification.
4. Dead bodies of plants and Animals are decomposed into nitrogenous wastes.
5. Nitrites are converted into nitrates by nitrification.
6. Nitrates are converted into gaseous nitrogen by denitrification [bacteria pseudomonas denitrificans , Thiobacillus bring about Denitrification].
7. Gaseous Nitrogen is made available for plants through nitrogen fixation by physical and biological methods.

Thus Nitrogen cycle goes on continuously in nature.

Unit 7

Plant growth regulators: Auxins, Gibberellins, Cytokinins, Ethylene ,ABA and their role in agriculture and horticulture.

4 Hrs

Phytohormones

The term ‘Hormones’ is derived from Greek word where ‘hormo’ means to ‘ Stimlae’. Thiamann coined the term. phytohormones are organic substances synthesised in minute concentration in one

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part of the plant body and transported to another part where they control growth and other physiological functions.

Classification of Phytohormones

Natural phyto hormones are classified into 2 groups. they are

- **Growth Promoters**:- The hormones which accelerates the growth and development in plants are called 'Growth promoters'. Ex: Auxins, Gibberlins, Cytokinins.
- **Growth Inhibitors**:- The hormones which retard or inhibit the growth and development in plants are called' Growth inhibitors.Ex: Ethylene , Abscissic acid.

Growth Promoters

Auxins (In Greek auxein means to grow).

- In 1880 Charles Darwin and his son Francis Darwin found that the sensation of unilateral illumination p by the coleoptile tip of Canary Grass.
- In 1910Boysen-Jensen showed that the sensation of phototropism picked up by coleoptile tip could be transmitted to sub-apical region.
- 1928 Went discovered that the hormone travelled from tip or apex towards the base. The growth promoting substance was named by him as auxin .
- In 1931 Kogl and Haagen-Smith isolated three chemicals from human urine.They were named as auxin a, auxin b, and hetero-auxin.
- In 1934 Kogl found that hetero-auxin is the real plant auxin and is chemically indole 3-acetic acid or IAA. It is an universal natural auxin It is also present in urine of human beings suffering from pellagra, a disease caused by deficiency of niacin.
- In 1934 Kogl discovered related chemicals indole 3-acetaldehyde, indole 3-acetonitrile, indole 3-butyric acid (IBA), phenyl acetic acid and 4-chloro indole acetic acid. All of them have auxin like activity.
- Auxin is synthesised in shoot apices, leaf primordia and developing seeds from amino acid tryptophan. Auxin passes from shoot tip to the region of elongation.
- The raw material used in synthesis of auxin is called auxin precursor. It is tryptophan for IAA.
- Certain compounds inhibit action of auxin. They are called anti-auxins, e.g., p-chloro phenoxy isobutyric acid (PCIB). TIBA (2, 3, 5 triiodobenzoic acid).

Synthetic Auxins:

Many synthetic auxins are also being manufactured. The important ones are 2: 4 D (2: 4-dichlorophenoxy acetic acid), 2 : 4 : 5-T (2 : 4 : 5-tri-chlorophenoxy acetic acid), IBA (indole 3-butyric acid), NAA (naphthalene acetic acid). MCPA (2-methyl 4- chloro-phenoxyacetic acid), Dicamba (2-

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methoxy 3-, 6-di-chlorobenzoic acid). IBA is both natural and synthetic. Synthetic auxins move in all directions inside plants.

Bioassay of Auxins: It is testing of a biological activity like growth response of a substance by employing a living material like plant or plant part.

1. Avena Curvature Test is based on experiments of Went. 10° curvature is produced by auxin concentration of 150 µg/litre at 25° C and 90% relative humidity. The test can measure auxin upto 300 pg/litre.

2. Root Growth Inhibition: Sterilized seeds of Cress are allowed to germinate on moist filter paper. As the roots reach a length of 1 cm, root lengths are measured. 50% of the seedlings are placed in a test solution while the remaining are allowed to grow over moist paper. Lengths of the roots are measured after 48 hours. It is seen that the seedlings placed in test solution show very little root growth while root growth is normal in control seedlings.

Functions of Auxins:

1. Respiration: Auxins stimulate respiration by increasing availability of respiratory substrate.

2. Metabolism: Application of auxin enhance metabolism due to mobilisation of plant resources.

3. Solutes: Auxins increase storage of solutes inside the cells.

4. Cell Enlargement: Cell enlargement is caused by solubilisation of carbohydrates, loosening of wall micro-fibrils, synthesis of more wall materials, increased membrane permeability and respiration.

5. Cambial Activity: Degree of cambial activity is directly proportional to auxin concentration. Auxin also controls xylem differentiation.

6. Cell Division: Auxin promotes division in the cells of vascular cambium.

7. Tissue Culture: In tissue culture, the development of callus or mass of undifferentiated cells is promoted by auxin.

8. Root Formation: Auxin promotes root initiation.

9. Apical Dominance: Apical dominance is the phenomenon by which presence of apical bud does not allow the nearby lateral buds to grow. When the apical bud is removed, the lateral buds sprout.

10. Inhibition of Abscission: Auxin delays abscission of young leaves and fruits. auxin promotes the abscission of mature or older leaves and fruits.

11. Tropic Movements: Differential distribution of in-dole 3-acetic acid produces phototropism and geotropism.

12. Sex: Auxins have a feminizing effect on some plants.

13. Seedless Fruits: Auxin promotes development of parthenocarpic fruits . e.g., Banana.

14. Ethylene: Higher concentration of IAA induces synthesis of ethylene.

15. Membrane Potential: It produces a negative potential on the cell membrane.

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Uses of Auxins:

1. **Rooting:** Auxins stimulate root formation on the stem cutting, e.g., IBA, IBA-alanine, NAA.
2. **Parthenocarp:** Application of auxins (e.g., IAA, IBA) and conjugate auxins (e.g., IBA-alanine) to unpollinated pistils make them develop into seedless fruits or parthenocarps which carry a better market price than the normal fruits having seeds.
3. **Weedicides:** Chemicals which kill weeds growing in the fields. Application of 2: 4-D and 2:4: 5-T removes broad leaved weeds in cereal crops and lawns because they do not affect mature monocotyledons while Dalapon (2-2 di-chloropropionic acid) kills grasses in broad leaved crops.
4. **Flowering:** NAA and 2, 4-D are often employed for inducing flowering in Litchi and Pineapple.
5. **Storage:** Methyl ester of NAA prevents the sprouting of Potato tubers kept in storage.
6. **Pre-Harvest Fruit Drop:** In low concentration 2, 4-D is useful in preventing pre-harvest fruit drop of Orange and Apple.
7. **Vegetable Crops:** Chlorophenoxy propionic acid enhances the quality of vegetable crops by preventing flower formation.
8. **Fruits:** Auxins enhance sweetening of fruits, e.g., IBA.
9. **Prevention of Lodging:** Naphthalene acetamide (NAAM) prevents lodging or falling of crop plants during windy season.
10. **Dwarf Shoots:** Application of naphthalene acetic acid increases the number of dwarf shoots and number of fruits.

Gibberellins

- Gibberellins known in Japan since 1800 where certain rice plants were found to suffer from bakane or bakanae (foolish seedling) disease.
- In 1918 Hori and Kurosawa in 1926 found that it is caused by a fungus, *Gibberella fujikori*.
- Yabuta in 1935 named it gibberellin. Yabuta also prepared crystalline form of gibberellin which actually consisted of six gibberellins.
- 1955 Brian et al isolated in pure form Gibberellic acid or GA₃. Cross in 1961 worked out the structure of gibberellic acid, GA₃.

It is chemically C₁₉H₂₂O₆. GA₃ is one of the most intensively studied gibberellin. Until now 125 different gibberellins have been identified. Many of them occur naturally in plants and fungi. *Gibberella fujikori* has as many as 15 gibberellins.

Bioassay of Gibberellins

1. **Dwarf Pea:** Seeds of dwarf pea are allowed to germinate till the formation of coleoptile. GA solution is applied to some seedlings. Others are kept as control. After 5 days, epicotyl length is measured. GA stimulates epicotyl growth with a concentration as low as 1 Nano gram.

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2. Barley Endosperm: Endosperms are detached from embryos, sterilized and allowed to remain in 1 ml of test solution for 1-2 days. There is a build-up of reducing sugars. The content of reducing sugar is proportional to gibberellin concentration. Reducing sugars are not formed in control experiment where endosperms are kept in plain water.

Functions of Gibberellins:

1. Stem and Leaf Growth: Gibberellins help in cell growth of stem, leaves and other aerial parts. Thus they increase the size of stem, leaves, flowers and fruits.

2. Dwarf Shoots: Gibberellins induce intermodal growth in some genetically dwarf varieties of plants like Pea and Maize.

3. Bolting: Gibberellins induce sub-apical meristem to develop faster. This causes elongation of reduced stem or bolting in case of rosette plants. Ex: Henbane, Cabbage, and root crops Ex: Radish

4. Dormancy: Gibberellins overcome the natural dormancy of buds, tubers, seeds, etc. and allow them to grow.

5. Seed Germination: During seed germination, gibberellins stimulate the production of some messenger RNAs and then hydrolytic enzymes like amylases, lipases ribonucleases and proteases in cereals. The enzymes solubilize the reserve food and it is transferred to embryo axis for its growth.

6. Fruit Development: They induce parthenocarpic fruit formation .e.g., Apple, Pear.

7. Flowering: They promote flowering in long day plants during non-inductive periods.

8. Vernalization: Vernalization or low temperature requirement of some plants can be replaced by gibberellins.

9. Sex Expression: Gibberellins promote the formation of male flowers on genetically female plants of Cannabis. They can also replace female flowers with male flowers on monoecious plants of cucurbits.

10. Curvatures: Gibberellins are responsible for phototropic and geotropic responses of shoot tips in Sunflower.

Uses of Gibberellins:

1. Fruit Growth: Application of gibberellins increases the number and size of several fruits, e.g., Grape, Tomato.

2. Parthenocarpy: Seedless fruits can be produced by application of gibberellins to un-pollinated flowers.

3. Malt: Gibberellins (e.g., GA₃) increase the yield of malt from barley grains.

4. Overcoming Dormancy: Gibberellins is used for breaking seed and bud dormancy. They induce germination of positively photoblastic seeds of Tobacco and Lettuce in complete darkness.

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5. Delayed Ripening: GA₇ delays senescence so that fruit can be left on the tree for longer period .(It extends period of marketing. Ripening of Citrus fruits can be delayed with the help of gibberellins. This is useful in storing the fruits).

6. Flowering: Gibberellins can be used in inducing off season flowering in many long day plants & plants requiring vernalisation.

7. Sugarcane: Spraying of sugarcane crop with gibberellins increases length of stem and yield of sugarcane to as much as 20 tonnes/acre.

8. Early Maturity: Juvenile conifers sprayed with mixture of GA₄ and GA₇ reach maturity quite early resulting in early seed production.

Cytokinins:

- Cytokinins is a plant growth hormones which are basic in nature, either amino purine or phenyl urea derivatives, that promote cytokinesis (= cell division) either alone or in conjunction with auxin.
- The first cytokinin was discovered from degraded autoclaved Herring sperm DNA by Miller 1955.
- It is called kinetin (6-furfuryl amino-purine). Kinetin does not occur naturally. It is a synthetic hormone.
- The first natural cytokinin was obtained from unripe maize grains or kernels by Letham (1964). It is known as zeatin (6-hydroxy 3-methyl trans 2-butenyl amino-purine).It also occurs in coconut milk.
- Up to now 18 types of cytokinins have been discovered. Some of them are constituents of transfer RNAs.
- Roots seem to be the major source of cytokinin synthesis. From roots the cytokinins pass upwardly through xylem.
- cytokinin synthesis also takes place in other areas where cell divisions are occurring like endosperm region of seeds, growing embryos and developing seeds, young fruits, developing shoots buds, etc. Coconut milk is a rich source of cytokinin.

Bioassay of Cytokinins:

1. Tobacco Pith Culture: Out of two tobacco pith cultures, one is supplied with cytokinin while the other is not. Increase in fresh weight of the tissue over the control is a measure of stimulation of cell divisions and hence cytokinin activity. The test can measure cytokinin concentration between 0.001-10 mg/litre. It takes 3-5 weeks.

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2. Retardation of Leaf Senescence: It is a rapid bioassay technique. Leaf discs are taken in two lots. In one lot cytokinin is provided. After 48-72 hours, the leaf discs are compared for chlorophyll content. Cytokinin retards the process of chlorophyll degradation. The test is sensitive in concentration of 1 pg/litre.

3. Excised Radish Cotyledon Expansion: The test was developed by Letham. Excised Radish cotyledons are measured and placed in test solution as well as ordinary water (as control). Enlargement of cotyledons is an indication of cytokinin activity.

Functions of Cytokinins:

1. Cell Division: Cytokinins are essential for cytokinesis .

2. Cell Elongation: Cytokinins cause cell elongation.

3. Morphogenesis: Cytokinins are essential for morphogenesis or differentiation of tissues and organs. In 1957 Skoog and Miller, reported that buds develop when cytokinins are in excess while roots are formed when cytokinins are in less.

4. Differentiation: Cytokinins induce formation of new leaves, chloroplasts in leaves, lateral shoot formation and adventitious shoot formation. They also bring about lignification and differentiation of inter-fascicular cambium.

5. Senescence (Richmond-Lang Effect): Cytokinins delay the senescence of leaves and other organs by mobilisation of nutrients.

6. Apical Dominance: It promotes apical dominance.

7. Seed Dormancy: They help to overcome seed dormancy of various types, including red light requirement of Lettuce and Tobacco seeds.

8. Resistance: Cytokinins increase resistance to high or low temperature and disease.

9. Phloem Transport: They help in phloem transport.

10. Accumulation of Salts: Cytokinins induce accumulation of salts inside the cells.

11. Flowering: Cytokinins can replace photoperiodic requirement of flowering..

12. Sex Expression: Cytokinins promote femaleness in flowers.

13. Parthenocarpy: In 1965 Crane reported induction of parthenocarpy through cytokinin treatment.

Uses of Cytokinins:

1. Tissue Culture: Cytokinins are essential for tissue culture as it responsible for cell division and also involved in morphogenesis. It is provided through the addition of coconut milk or yeast extract medium.

2. Shelf Life: Application of cytokinins to marketed vegetables can keep them fresh for several days. (Shelf life of cut shoots and flowers is prolonged).

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3. Resistance: Cytokinin helps plants in developing resistance to pathogens and extremes of temperature.

4. Overcoming Senescence: Cytokinins delay senescence of intact plant parts.

Growth Inhibitors

I.Ethylene

- Ethylene is a gaseous hormone which stimulates transverse or isodiametric growth but retards the longitudinal one.
- In 1910 Cousins found that ripe oranges produced a volatile substance that promoted ripening of unripe bananas.
- In 1934 R. Gane found that the ripening causing volatile substance was ethylene.
- In 1935 Crocker recognised Ethylene as a plant hormone.
- Ethylene is produced in plants from the amino acid methionine. It is formed in almost all plant parts— roots, leaves flowers, fruits, and seeds.

Functions of Ethylene:

1. Growth: Ethylene inhibits longitudinal growth but stimulates transverse or horizontal growth and swelling of axis.

2. Gravity: It decreases the sensitivity to gravity. Roots become Apo-geotropic while stems turn positively geotropic. Leaves and flowers undergo drooping. The phenomenon is called epinasty.

3. Senescence & Abscission: It brings the senescence of leaves and flowers. Abscission of leaves, flowers, fruits.

5. Apical Dominance: Ethylene promotes apical dominance and prolongs dormancy of lateral buds.

6. Breaking of Dormancy: It breaks the dormancy of buds, seeds and storage organs.

7. Abscisic Acid: Abscisic acid is formed in the leaves under conditions of water stress is mediated through ethylene.

8. Growth of Rice Seedling: Ethylene promotes rapid elongation of leaf bases and internodes in deep water rice plants. As a result leaves remain above water.

9. Root Initiation: In low concentration ethylene helps in root initiation, growth of lateral roots and root hairs.

10. Fruit Ripening: It aids in ripening of climacteric fruits and dehiscence of dry fruits. Climacteric fruits are fleshy fruits which show a sudden sharp rise of respiration rate at the time of ripening (respiratory climacteric). They are usually transported in green or unripe stage. Ethylene is used to induce artificial ripening of these fruits. Ex: Apple, Mango, Banana, etc.

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11. Flowering: It stimulates flowering in Pineapple, Mango and also causes fading of flowers. This helps in synchronizing fruit set.

12. Sex Expression: Ethylene has a feminizing effect on sex expression. The genetically male plants of Cannabis can be induced to produce female flowers in the presence of ethylene.

Uses of Ethylene

The uses of ethylene are as follows:-

1. Fruit Ripening: Ethylene lamps are now used for stimulating colour development and ripening of some fleshy fruits, e.g., Banana, Mango, Apple, and Tomato.

2. Feminising Effect: External supply of very small quantity of ethylene increases the number of female flowers and hence fruits in Cucumber.

3. Sprouting of Storage Organs: Rhizomes, corms, tubers, seeds and other storage organs can be made to sprout early by exposing them to ethylene.

4. Thinning: Better growth of fruits excess flowers and young fruits are thinned with the help of ethylene. Ex: Cotton, Cherry, and Walnut.

II. Abscisic Acid

- Abscisic Acid is also called stress hormone because the production of hormone is stimulated by drought, water logging and other adverse environmental conditions.
- In 1963 Addicott isolated this hormone from Cotton bolls. Abscisic acid is known as Dormin as it induces dormancy in buds, underground stems and seeds.
- Abscisic acid is a mildly acidic growth hormone which functions as a general growth inhibitor by counteracting other hormones such as auxin, gibberellins, and cytokinins or reactions mediated by them.
- It is produced in many parts of the plants and abundantly inside the chloroplasts of green cells and later transports to all parts of the plant through diffusion and through phloem and xylem.

Functions of Abscisic Acid

1. Bud Dormancy: Abscisic acid induces dormancy of buds..

2. Seed Dormancy: It causes seed dormancy .

3. Stoppage of Cambium Activity: Abscisic acid stops mitosis in vascular cambium towards the approach of winter.

4. Abscission: Abscisic acid promotes abscission of flowers and fruits.

5. Leaf Senescence: Its excessive presence stops protein and RNA synthesis in the leaves and stimulates their senescence.

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- 6. Transpiration:** Abscisic acid is rapidly synthesised during desiccation and other stresses. it results in closure of stomata and prevents transpiration.
- 7. Resistance:** Abscisic acid increases resistance of plants to cold and other types of stresses. It is, therefore, also known as stress hormone.
- 8. Starch Hydrolysis:** Abscisic acid inhibits gibberellin mediated amylase formation during germination of cereal grains.
- 9. Flowering:** In small quantities, abscisic acid is known to promote flowering in some short day plants
Ex: Strawberry.
- 10. Rooting:** It promotes rooting of stem cuttings Ex:Bean.
- 11. Membrane Potential:** ABA induces a positive surface potential on cell membrane.
- 13. Controlled Growth:** It is antagonist to gibberellins and counteracts the effect of other growth promoting hormones.

Uses of Abscisic Acid

- 1. Antitranspirant:** Application of minute quantity of abscisic acid to leaves shall reduce transpiration to a great extent through partial closure of stomata. It conserves water and reduces the requirement of irrigation.
- 2. Flowering:** It is useful in introducing flowering in some short day plants kept under un-favourable photoperiods.
- 3. Rooting:** Use of abscisic acid promotes rooting in many stem cuttings.
- 4. Dormancy:** Abscisic acid is used in prolonging dormancy of buds, storage organs and seeds.

Unit 8

Plant response to light and temperature

Photoperiodism , Phytochromes, Florigen concept, Vernalization. 4 Hrs

Photoperiodism: K.S.Gitanjali,SSCASC,TUMKUR

Introduction:

The plants in order to flower require a certain day length i.e., the relative length of day and night which is called as photoperiod. The response of plants to the photoperiod for flowering is called as **photoperiodism**.

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The phenomenon of photoperiodism was first discovered by Garner and Allard (1920, 22). He observed that the Biloxi variety of Soybeans (*Glycine max*) and 'Maryland Mammoth' variety of tobacco (*Nicotiana tabacum*) could be made to flower only when the daily exposure to the light was reduced below a certain critical duration. After many complex experiments concluded that **'the relative length of the day is a factor of the first importance in the growth and development of plants'**.

Depending upon the duration of the photoperiod, they classified plants into three categories.

(1) Short Day Plants (SDP):

These plants require a relatively short day light period (usually 8-10 hours) and a continuous dark period of about 14-16 hours for flowering. These plants are also known as long-night-plants. Ex:- Maryland Mammoth variety of tobacco (*Nicotiana tabacum*) Biloxi variety of Soybeans (*Glycine max*), Cocklebur (*Xanthium pennsylvanicum*).

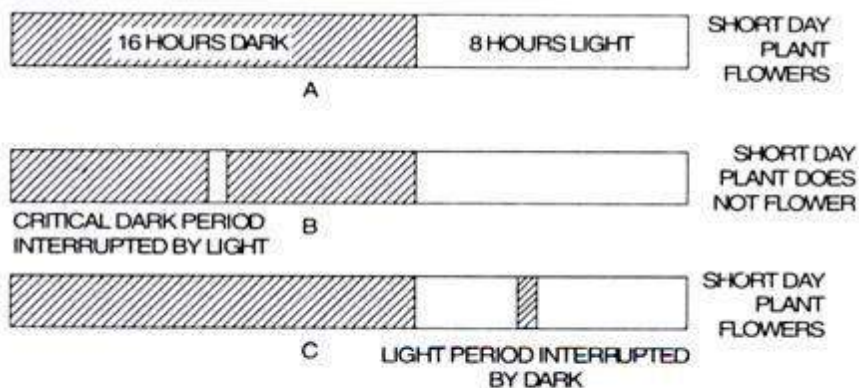


Fig. 18.1. Effect of a brief exposure of red light during dark and interruption of light period by dark on flowering in a short day plant.

- i. In short day plants the dark period is critical and must be continuous. If this dark period is interrupted even with a brief exposure of red light, it will not flower.
- ii. The inhibitory effect of red light can be overcome by a subsequent exposure with far-red light (730-735 nm wavelengths).
- iii. Interruption of the light period by dark does not have an inhibitory effect on flowering in short day plants (Fig. 18.1 C).
- iv. Prolongation of the continuous dark period initiates early flowering in short day plants.

(2) Long Day Plants (LDP):

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These plants require a longer day light period (usually 14-16 hours) in a 24 hours cycle for subsequent flowering. These plants are also called as **short night plants** .Ex: Spinacea (spinach) Beta vulgaris (Sugar beet).

- i. In long day plants the light period is critical.
- ii. A brief exposure in the dark period or the prolongation of the light period stimulates flowering in long day plants.

(3) Day Neutral Plants:

These plants flower in all photoperiods ranging from 5 hours to 24 hours continuous exposure. Ex: Tomato, cotton, sunflower, cucumber and certain varieties of peas and tobacco.

During recent years certain intermediate categories of plants have also been recognised. They are,

A)Long Short Day Plants:

These are short day plants but must be exposed to long days during early periods of growth for subsequent flowering. Some of the examples of these plants are certain species of Bryophyllum.

B)Short-Long Day Plants:

These are long day plants but must be exposed to short days during early periods of growth for subsequent flowering. Some of the examples of these plants are certain varieties of wheat (Triticum) and rye (Secale).

Photoperiodic Induction:

Plants may require one or more inductive cycles for flowering. An appropriate photoperiod in 24 hours cycle constitutes one inductive cycle. If a plant which has received sufficient inductive cycles is subsequently placed under un-favourable photoperiods, it will still flower. Flowering will also occur if a plant receives inductive cycles after intervals of un-favourable photoperiods (i.e., discontinuous inductive cycles). This persistence of photoperiodic after effect is called as photoperiodic induction.

- i. An increase in the number of inductive cycles results in early flowering of the plant. For instance Xanthium (a short day plant) requires only one inductive cycle and normally flowers after about 64 days. It can be made to flower even after 13 days if it has received 4-8 inductive cycles. In such cases the number of flowers is also increased.

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ii. Continuous inductive cycles promote early flowering than discontinuous inductive cycles. Ex: Biloxi soybean (SDP) — 2 inductive cycles; *Salvia occidentalis* (SDP) — 17 inductive cycles; *Plantago lanceolata* (LDP)—25 inductive cycles.

Photoperiodic Stimulus and Presence of a Floral Hormone:

The photoperiodic stimulus is perceived by the leaves. As a result, a floral hormone is produced in the leaves which is then trans located to the apical tip, subsequently causing the initiation of floral primordia.

That the photoperiodic stimulus is perceived by the leaves can be shown by simple experiments on cocklebur (*Xanthium pennsylvanicum*), a short day plant. Cocklebur plant will flower if it has previously been kept under short-day conditions. If the plant is defoliated and then kept under short day condition, it will not flower. Flowering will also occur even if all the leaves of the plant except one leaf have been removed .

If a cocklebur plant whether intact or defoliated, is kept under long day conditions it will not flower . But, if even one of its leaves is exposed to short day condition and the rest are under long day photoperiods, flowering will occur .

The photoperiodic stimulus can be transmitted from one branch of the plant to another branch. For example, if in a two branched cocklebur plant one branch is exposed to short day and other to long day photo period, flowering occurs on both the branches .

Flowering also occurs if one branch is kept under long day conditions and other branch from which all the leaves except one have been removed is exposed to short day condition However, if one branch is exposed to long photoperiod and the other has been defoliated under short day condition, flowering will not occur in any of the branches .

Nature of the Floral Hormone:

Floral hormone has been named as florigen is not very clear. But it is quite evident that this hormone is a material substance which can be trans located from leaves to the apical tips situated at other parts of the plant resulting in flowering. 'florigen' to be a macromolecule like other plant growth hormones . This macromolecule may possibly be a RNA or protein molecule which is trans located from the leaf to the apical tips (or meristems) via phloem in photo-induced plants. (Corbesier and Coupland, 2005).

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Grafting experiments in cocklebur plants have even proved that the floral hormone can be translocated from one plant to another. For example, if one branched cocklebur plant, which has been exposed to short day conditions is grafted to another cocklebur plant kept under long day conditions, flowering occurs on both the plants .

Obviously the floral hormone has been transmitted to the receptor plant through graft union. But if a cocklebur plant is grafted to another similar plant both of which have been kept under long day conditions, flowering will not occur on either of the two plants .It has also been indicated that the floral hormone may be identical in short-day and long- day plants. For example, grafting experiments between certain long-day plants and short-day plants have shown that flowering occurs on both the plants even if one of them has been kept under non-inductive photoperiods.

Phytochrome:

Proteinaceous pigment that inhibits flowering in Short day plants and Stimulates flowering in Long day plants by interruption in dark period is called “Phytochrome”.

- Vierstra and Quail in 1983 reported Successful purification of intact native phytochrome from etiolated oat seedlings.
- The native phytochrome is a **soluble protein** with a molecular weight of about 250 kDa. It's a **homodimer** of two identical polypeptides each with a molecular weight of about 125 kDa.
- Each polypeptide has a prosthetic group called as **chromophore** which is covalently linked to the polypeptide via a sulphur atom (Thioether Linkage) in the cysteine residue of the polypeptide. The protein part of the phytochrome is called as **apoprotein**. Apoprotein along with chromophore constitute holoprotein

i. The pigment phytochrome exists in two different forms:

(i) **Red light** absorbing form which is designated as P_R and

(ii) **Far-red light** absorbing form which is designated as P_{FR} .

ii. These two forms of the pigment are photo chemically inter convertible.

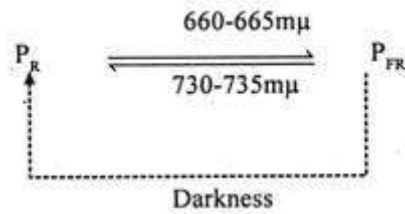
iii. When P_R form of the pigment absorbs red light (660-665mp), it is converted into P_{FR} form.

iv. When P_{FR} form of the pigment absorbs far-red light (730-735mp), it is converted into P_R form.

v. The P_{FR} form of the pigment gradually **changes into P_R form in dark.**

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- Brief exposure with red light during critical dark period inhibits flowering in short-day plants.
- This inhibitory effect can be reversed by a subsequent exposure with far-red light.
- The prolongation of the critical light period or the interruption of the dark period stimulates flowering in long-day plants.
- During the day the P_{FR} form of the pigments is accumulated in the plant which is inhibitory to flowering in short-day plants but is stimulatory in long-day plants.
- During critical dark period in short-day plants, this form gradually changes into P_R form resulting in flowering. A brief exposure with red light will convert this form again into P_R form thus inhibiting flowering.
- Reversal of the inhibitory effect of red light during critical dark period in SDP by subsequent far-red light exposure is because the P_{FR} form after absorbing far-red light (730-735mμ) will again be converted back into P_R form.

TYPES OF PHYTOCHROMES IN PLANTS

There are two major types of phytochromes in plants, (i) type I and (ii) type II.

The type I predominates in etiolated seedlings. Type I phytochrome is encoded by PHY A gene while type II is encoded by PHY B, PHY C, PHY D and PHY E genes.

The type II in green plants and seeds (such as oat seeds). There are minor differences in molecular weight and spectral properties of these two types of phytochromes.

Importance of Photoperiodism:

(i) The knowledge of photoperiodism has been of great practical importance in hybridisation experiments.

(ii) Although the floral hormone 'florigen' has not yet been isolated, the isolation and characterization of this hormone will be of utmost economic importance.

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(iii) The phenomenon of photoperiodism is an excellent example of physiological preconditioning where an external factor i.e., the photoperiodic stimulus, induces some physiological changes in the plant .

Phytochrome Mediated Photo responses in Plants:

1. Photoperiodism.2. Seed germination.3. Elongation of leaf, petiole, stem. 4. Hypocotyl hook unfolding.5. Unfolding of grass leaf. ,6. Sex expression. 7. Bud dormancy.8. Plastid morphology.9. Plastid orientation.11. Rhizome formation.12. Bulb formation.13. Leaf abscission.14. Epinasty.15. Succulency16. Enlargement of cotyledons.17. Hair formation along cotyledons.18. Formation of leaf primordia. 19. lower induction.20. Differentiation of primary leaves.21. Formation of tracheary elements.22. Differentiation of stomata.23. Change in rate of cell respiration.24. Formation of phenylalanine deaminase.25. Synthesis of anthocyanins.26. Increases in protein synthesis.27. Increase in RNA synthesis.28. Changes in the rate of fat degradation.29. Changes in the rate of degradation of reserve proteins.30. Auxin catabolism.31. Incorporation of sucrose into plumular tissue.32. Permeability of cell membranes.33. Lipoxygenase metabolism.

Vernalization:

K.S.Gitanjali,SSCASC,TUMKUR

Introduction;

Many plants do not come to flower before they experience a low temperature. These plants remain vegetative during the warm season, receive low temperature during winter, grow further and then bear flowers and fruits. Requirement of low temperature prevents precocious reproductive development in autumn.

It allows the plant to reach vegetative maturity before reproduction can occur. The condition occurs in winter varieties of some annual food plants (e.g., Wheat, Barley, and Rye), some biennial (e.g., Cabbage, Sugar beet, Carrot) and perennial plants (e.g., Chrysanthemum).

The annual winter plants also possess spring varieties. The spring varieties are planted in spring. They come to flower and bear fruits prior to end of growing season.

If the winter varieties are sown similarly, they fail to flower and produce fruits before the end of growing season. They are planted in autumn, form seedlings in which form they cover winter. The seedlings resume growth in spring. They bear flowers and fruits in summer.

Definition:

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Lysenko (1928), a Russian worker that **the cold requiring annual and biennial plants can be made to flower in one growing season by providing low temperature treatment to young plants or moistened seeds.** He called the effect of this chilling treatment as “**vernalization**”. Vernalization is, therefore, a process of shortening of the juvenile or vegetative phase and hastening flowering by a previous cold treatment.

Site for Vernalization:

The stimulus of vernalization is perceived only by the meristematic cells, e.g., shoot tip, embryo tips, root apex, developing leaves, etc.

Requirements of Vernalization:

(i) Low Temperature: Low temperature required for vernalization is usually 0° — 5° . It is 3° — 17° in case of biennial Henbane (*Hyoscyamus niger*). Low temperature treatment should not be immediately followed by very high temperature (about 40°C) otherwise the effect of vernalization is lost. The phenomenon is called de-vernalization.

(ii) Period of Low Temp. Treatment: It varies from a few hours to a few days.

(iii) Actively Dividing Cells: Vernalization does not occur in dry seeds. The seeds must be germinated so that they contain an active embryo. For this the seeds are moistened before exposing them to low temperature. In whole plants, an active meristem is required.

(iv) Water: Proper protoplasmic hydration is must for perceiving the stimulus of vernalization. **(v) Aerobic Respiration and (vi) Proper Nourishment.**

Mechanism of Vernalization:

The stimulus received by the actively dividing cells of shoot or embryo tip travels to all parts of the plant and prepare it to flower. The stimulus has been named as vernalin. It can be passed from one plant to another through grafting in case of Henbane but not in others. However, the chemical has not been separated. In some plants cold treatment can be replaced by gibberellins.

Vernalization prepares the plant to flower. The induction of flowering depends upon the presence of other favourable conditions. Photoperiodism, however, not only prepares the plant to flower but also brings about flowering. Thus, Henbane is a long-day plant which also requires cold treatment. Unless and until both are provided the plant will not come to flower,

Importance of Vernalization:

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(i) Vernalization can help in shortening the juvenile or vegetative period of plant and bring about early flowering. It is not only applicable to temperate plants but also to some tropical plants, e.g., Wheat, Rice, Millets, Cotton,

(ii) It increases yield, resistance to cold and diseases, and

(iii) Kernel wrinkles of Triticale can be removed by vernalization.

Unit 9

Dormancy: a brief account of seed dormancy (1 Hour)

Seed Dormancy:

K.S.Gitanjali,SSCASC,TUMKUR

Dormancy is a delaying mechanism which prevents germination of seed.

DEFINITION:-Seed dormancy is the innate inhibition of germination of a viable seed even when placed in most favourable environment for germination.

Bewley and Black (1994) have divided seed dormancy into two categories, seed coat based and embryo based. Germination inhibitors occur in both.

Types of Seed Dormancy:

Harper (1977) recognizes three types of seed dormancy depending on the how each of them arises: viz., innate, enforced and induced.

The seed dormancy is of following types:

i. Innate dormancy:

Innate dormancy may be imposed chemically by the presence of inhibitory compounds either in the seed coat or in the embryo. In some cases environmental conditions such as chilling, fluctuating temperature of specific photoperiods,

ii. Enforced dormancy:

Enforced dormancy occurs when seed is deprived of its requirements for germination, for example, by the absence of sufficient moisture, oxygen, light or a suitable temperature.

iii. Induced dormancy:

Induced dormancy is caused by the unfavourable conditions..

Causes of Seed Dormancy:-

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Plant physiology and Metabolism

1. Immaturity of Embryo: Embryo is immature at the time of seed shedding. The seed will remain dormant till the embryo becomes mature, e.g., *Anemone nemorosa*, *Ranunculus ficaria*.

2. light: Light sensitive seeds do not germinate in absence of light; whereas light hard seeds do not germinate on the exposure to light.

3. Temperature: Generally the low temperatures promote and high temperatures inhibit the germination. In the Indian desert many weed seeds must pass through very high temperature (60°-70C) of sand in day and at the same time low temperatures (5°-10°C) of night before they are stimulated for germination.

4. Impermeable Seed Coat:- The seed coat is impermeable to water and gases, e.g., Apple, *Chenopodium*.

5. Hard Seed Coat:- The seed coat is mechanically resistant and does not allow the embryo to grow, e.g., *Amaranthus*, *Lepidium*.

6. Germination Inhibitors:- Germination inhibitors causing seed dormancy are abscisic acid, phenolic acid, ferulic acid, coumarin, short fatty acids and cyanogenic chemicals. They occur in the seed coats and cotyledons of the embryos. e.g., Apple, Peach, Ash, *Cucurbita*, *Iris*, *Xanthium*.

METHODS OF BREAKING DORMANCY

Seed dormancy can be broken and make seed to germinate by 2 methods . They are :- I) Natural method and II) Artificial method

I) Natural methods to Break Seed Dormancy:

In nature seed dormancy is broken automatically due to:

- (i) Development of growth hormones to counter growth inhibitors,
- (ii) Leaching of germination inhibitors,
- (iii) Maturation and after-ripening of embryo,
- iv) Weakening of impermeable and tough seed coats by microbial action, abrasion, passage through digestive tract of animals, etc.

II) Artificial Breaking of Seed Dormancy:

1. Scarification:- Hard, impermeable seed coat is weakened or ruptured by filing, chipping, hot water and chemicals.

2. Stratification:- Seeds are moistened and exposed to oxygen for variable period at low or high temperature.

3. Counteracting Inhibitors:- Inhibitors are destroyed by dipping seeds in KNO_3 , thiourea, ethylene chlorohydrin and gibberellin.

4. Shaking and Pressure:- Vigorous shaking and hydraulic pressure are used to weaken seed coats.

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Importance of Seed Dormancy:

- 1. Perennation:**-Seed dormancy allows seeds to pass through drought, cold and other un-favourable conditions.
- 2. Dispersal:-** It is essential for dispersal of seeds.
- 3. Germination under Favourable Conditions:-**Seeds germinate only when sufficient water is available to leach out inhibitors and soften the seed coats.
- 4. Storage:-** Dormancy is responsible to store grains, pulses and other edibles for making them available throughout the year and transport to the areas of deficiency.

Unit 10 (2 Hrs)

Plant movements: (phototropism, geotropism, hydrotropism and seismonasty)

PLANT MOVEMENTS

Movement of plant body from one place to another or the reorientation of plant organs is called plant movements.

- Movement of entire plant body from **one place to another place** is called **movement of locomotion.**
- **Reorientation** of various organs in the plant which is anchored to the soil is called **movement of curvature.**
- Movement of curvature may be **accompanied by growth** called **Growth movements** or **not accompanied by growth** called **Variation movements.**
- When the movements are not induced by **external stimuli** it is called **autonomic movement.**
- When the movements are induced by **unidirectional external stimuli** it is called **Paratonic movement.**

CLASSIFICATION OF PLANT MOVEMENTS

PLANT MOVEMENTS

1. MOVEMENTS OF LOCOMOTION

AUTONOMIC:

- a) Ciliary
- b) Cyclosis
- a) Phototropic b) Geotropic, c) Hydrotropic
- a) Photactic
- b) Chemotactic
- c) Termotactic

2. MOVEMENTS OF CURVATURE

I. GROWTH MOVEMENT

AUTONOMIC : a) Nastic b) Nutational

PARATONIC:

PARATONIC

II .VARIATION MOVEMENT

AUTONOMIC : a) Gyration

PARATONIC: a) Nyctinastic ,b) Seismonastic

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Plant physiology and Metabolism

PARATONIC MOVEMENT OF GROWTH

- Growth Movement due to unilateral external stimuli such as light, Gravity, water is called "Tropism".

PHOTOTROPISM



a)PHOTOTROPISM : Movements in response to unilateral light stimulus is called Phototropic movement or Phototropism. Ex: Stem tips are positively phototropic, roots are negatively phototropic .

b)GEOTROPISM:

Movements in response to gravity, is called Geotropism. Ex: Root is positively geotropic, primary stem is negatively Geotropic.

c)HYDROTROPISM:

Movements in response to external stimulus of water is called Hydrotropism



d)THIGMOTROPISM

Movements in response to stimulus of touch is called Thigmotropism. Ex: Tendril

VARIATION MOVEMENTS

The movement of plant organ which is not associated with growth is called variation movement.

a) GYRATION: Indian telegraph plant shows Trifoliate leaves ,one terminal leaf let is larger and two lateral leaf lets are smaller. During day time smaller lateral leaf lets show peculiar upward and

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MOVEMENT OF CURVATURE

Reorientation of plant organs accompanied by **growth** is called **Growth movements**. Reorientation of plant organs not accompanied by growth is called **Variation Movements**.

Autonomic Nastic movement

1. **NASTIC MOVEMENTS:** Movement due to **differential growth** in bifacial organs such as leaves, sepals, petals.

a) **Hyponastic movement:** when more growth takes place on its **lower surface** of sepals, it is called Hyponastic movement.



b) **Epinastic movement** : when more growth takes place on **upper surface** of petal , bud opens . It is called Epinastic movement .

2. **NUTATIONAL MOVEMENTS**

Growth due to continuous change in position of growing organ is called **Nutation**, because of slowness in growth it can't be observed by naked eye.

Ex : Stem, Root, Tendril, Pedicel.

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Plant physiology and Metabolism

Practical – VII

Credits- 2

1. Determination of osmotic potential of plant cell sap by plasmolytic method.
2. Determination of rate of transpiration by Ganong's potometer.
3. Determination of stomatal index by Quickfix method.
5. Determination of Rate of Photosynthesis at different wave lengths of light.
6. Determination of Rate of Photosynthesis at different Concentrations of CO_2 .
7. Separation of Photosynthetic pigments by paper chromatography and measurement of R_f values

Demonstration experiments

1. Hydroponics
2. Study of any two mineral deficiency Symptoms.
3. Separation of photosynthetic pigments by solvent wash method.
4. Kuhne's experiment
5. Determination of Catalase activity.
6. Phototropism, geotropism and hydrotropism.

Suggested Readings

1. Taiz, L., Zeiger, E., (2010). Plant Physiology. Sinauer Associates Inc., U.S.A
5th Edition.
2. Hopkins, W.G., Huner, N.P., (2009). Introduction to Plant Physiology. John Wiley & Sons, U.S.A. 4th Edition.
3. Bajracharya, D., (1999). Experiments in Plant Physiology- A Laboratory Manual. Narosa Publishing House, New Delhi.
4. Jain. Plant Physiology
5. Salisbury and Ross. Plant Physiology Wardsworth pub California. USA
6. H. Srivastava. Plant Physiology, S. Chand and Co.
7. Bidwell. RGS Plant Physiology.(Revised Edition)
8. Devlin and Witham. Plant Physiology
- 9, Verma Sk & Verma Mohit. T.B. of Plant Physiology, Biochemistry and Biotechnology, S. Chand & Co.
10. P.S. Gill Plant Physiology. S. Chand & Co.

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Plant physiology and Metabolism

11. Leninger AC. Principles of Biochemistry:

SSCASC

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Plant physiology and Metabolism

Practical Question Paper– paper –VII

Time: 3 Hrs Max.marks: 50

1. Separate the photosynthetic pigments from the sample 'A' by paper chromatography and measure their Rf values. 15
2. Determine the Osmotic potential of the cell sap by Plasmolytic method / Stomatal index of the material 'B'. 10
3. Comment on the experiments C, D and E 15
4. Viva voce 5
5. Class records 5

Scheme of valuation.

1. Requiriments – 1 mark, Principle – 2 marks, Procedure – 5 marks, Conduction – 4 marks, Rf value- 3 marks.
2. Requirements – 1 mark, Principle- 1mark, , Procedure- 3 marks, Conduction – 4 marks, Calculation & result – 1 mark.
3. Identification – 1 mark, Principle -1mark, diagram – 1mark , comment – 2 marks.

Experiments : Any two of the following

1. Ganong's potometer
2. Hydroponics
3. Mineral deficiency Symptoms (specimen / photocopy)
4. Rate of photosynthesis at different wavelengths of light / different Concentrations of Co₂ .
5. Kuhne's experiment
6. Phototropism
7. Geotropism
8. Hydrotropism
4. Viva-voce related to experiments given in the practical examination.
5. Class records

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Plant physiology and Metabolism

References:

1. Introduction to plant physiology: William G. Hopkins. John wiley and sons, Inc.
2. Cell biology: Gerald Karp.
3. T.B. of plant physiology , Biochemistry and Biotechnology : Verma SK & Verma Mohit. S.chand & co
4. Principles of Biochemistry: Leninger AC.
5. Plant physiology: RGS. Bidwell.
6. Plant physiology: Salisbury FB & Rose C.W.Wardsworth pub California. USA
7. Plant physiology: H. Srivastava. S.chand& co.
8. Fundamentals of plant physiology: V.K. Jain. S.chand & co

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Plant physiology and Metabolism

BOTANY –PAPER -VIII

Molecular Biology, Genetic engineering, Bioinformatics and Biotechnology

Theory -90 Marks Credits-3 45 Hrs

Unit-1; Molecular biology

Genetic material: Introduction, identification of genetic material (Griffith's and Avery's experiments, Hershey-Chase experiment) ,

Chemical nature of genetic material: Nucleotides, nucleosides

DNA: structure, replication (Semi-Conservative and Rolling circle model)

RNA: Structure, genetic RNA and non genetic RNA (mRNA, rRNA, tRNA),

Biosynthesis of proteins: Genetic code, Transcription, RNA Splicing, Translation and Polysomes.

Regulation of gene expression: Prokaryotes: (Lac- Operon) and Eukaryotes (Britten and Davidson's model). **16 Hrs**

Unit-2: Bio-molecular Techniques

Blotting techniques: Northern, Southern and Western Blotting,

DNA finger printing; DNA sequencing (Sanger's method), PCR **6 Hrs**

Unit -3: Genetic engineering:

A concise account of methods used in recombinant DNA technology.

Tools of rDNA technology: Plasmids (PBR322, PUC 18, Ti-plasmid), Restriction endonuclease, DNA ligase, and Bioreactor.

Genomic and cDNA libraries, screening of genomic library.

Applications of Genetic Engineering technology in agriculture

(Transgenic plants- Bt-cotton, golden rice), in medicine (insulin synthesis, gene therapy), in environment (bioremediation and bio-mining). **12Hrs**

Unit-4: Bioinformatics

Introduction, Aim and scope

Biological Databases: DNA database and protein databases.

A brief account of NCBI, DNA Data Bank of Japan (DDBJ) and Protein Information Resource (PIR) **5 Hrs**

Unit 5; Biotechnology:

Fermentation technology: Production of Ethyl alcohol, production of antibiotics (Penicillin), production of single cell protein (*Spirulina*) :

Environmental technology: Waste water treatment process: primary, secondary and advanced treatment of sewage (domestic waste water), **6 Hrs**

VI SEMESTER PAPER-VII UNIT-2

K S Gitanjali SSCASC

BOTANY PAPER- VII

K S Geethanjali SSCASC

MOLECULAR BIOLOGY

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The branch of Biology that deals with the study of structure and functions of Bio molecules in the living body is called Molecular Biology . In 1945 william Astbury coined the term molecular biology. Organic components of the cell such as Proteins, Carbohydrates ,Lipids and Nucleic acids form Bio molecules.

Identification of Genetic material

A genetic material has to be stable in its structure and have self replicating capacity to transfer the biological information from one generation to the next. DNA posses all these characteristics .Hence DNA is regarded as genetic material.

DNA as Genetic material experiment:

Griffth 's

Fredrich griffth , a British medical officer in 1928 while working on pathogenicity of streptococcus pneumonia (it Cause pneumonia in mammals) observed in two strains of bacteria. They are :

- 1) **smooth strain(S)** : It has polysaccharide capsule.
- 2) **Rough strains(R)** : It lacks polysaccharide capsule.it produce rough colonies in culture and it is nonpathogenic.

Griffth injected heat killed' **S**' strain into mice ,it did not cause pneumonia .When such heat killed strain was mixed with living' **R**' strain and cultured ,instead of R colonies **S colonies were formed** .When it was injected into mice they died of pneumonia. Autopsis showed presence of' **S**' strain . According to Griffth some chemical present in' **S**' strain is responsible for change of' **R**' strain into' **S**' strain he called it as "**Transforming principle**".

Avery experiment

Oswald T avery,McLeod,McCarty in 1944 conducted experiment and proved that "**Transforming principle**" Is DNA. They conducted experiment on mice with Streptococcus pneumonia as follows :-

- 1) A culture of **R strain** produced rough colonies .When it was injected into mouse it did not cause pneumonia .

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- 2) A culture of **S strain** produced smooth colonies when injected into a mice it caused pneumonia .
- 3) A culture of heat killed **S strain** did not form any colony.
- 4) A culture of **heat killed' S' strain mixed with living' R' strain** produced smooth colonies of ' S' strain instead of ' R' strain colonies,
- 5) Heat killed **S strain were treated with** an enzyme a **protease** and then cultured with ' R' strain ,S strain colonies were formed .
- 6) Heat **killed S strain were treated with** an enzyme Protease and then cultured with R strain 'S' strain colonies were formed,
- 7) Heat **killed S strain were treated with** an enzyme DNAase then cultured with 'R 'strain ,only R'colonies were formed,Transformation was absent.
- 8) These experiments clearly proved that **the Transformation principle is DNA** and not RNA or protein.

Deoxy Ribose Nucleic acid

DNA is a molecule of heredity .It functions as genes in all organisms except Viruses. In Eukaryotic nucleus , DNA is in the form of double helix . In Bacteria , Mitochondria and Plastids DNA molecules are circular.

CHEMICAL COMPOSITION OF DNA

DNA is made up of 3 chemical components .namely Sugar, Phosphoric acid , Nitrogenous bases.

SUGAR

The sugar present in DNA is De oxy Ribose. It is a pentose sugar .It is a 5 carbon sugar . At carbon no 2 position one oxygen atom atom is less than Ribose sugar .

Phosphoric acid

Phosphoric acid is an inorganic acid .In nucleic acid it is alternately arranged with pentose sugar . 'p' is linked to 3 'c' atom of one pentose sugar and 5 'C' atom of another pentose sugar by phosphor di ester bonds .

NITROGEN BASES

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These are Nitrogen containing organic compounds .They are of 2 types namely:-

- 1) **Purines**
- 2) **Pyrimidines**

Purines are two ringed nitrogen compounds. They are Adenine and Guanine. **Pyrimidines** are single ringed Nitrogen compounds .They are Thymine, Cytosine and Uracil . Uracil is absent in DNA.

Nucleotide and Nucleoside

Nucleotide :- Nucleotides are made up of 3 components. They are Nitrogen base ,pentose sugar and phosphoric acid .

Nitrogen base may be purines like Adenine ,Guanine and Pyrimidines like Guanine , Cytosine ,Uracil. Sugar may be Deoxy ribose or Ribose. Accordingly they are grouped into deoxy ribose nucleotide and ribonucleotide.

Nucleotides are named according to Purines and pyrimidines as follows:-

Nitrogen base	Nucleotide
Adenine	Adenilic acid or Adenosine monophosphate
Thymine	Thymidilic acid or Thymidine Monophosphate
Cytosine	Cytidilic acid or cytosine Monophosphate
Uracil	Uridylic acid or uridine Monophosphate .

Nucleotides may occur in tissues freely as ATP(Adenosine di phosphate) and ATP(Adenosine tri phosphate).

Significance of Nucleotides

1. Nucleotides form main components of Nucleic acids.
2. The deoxy ribonucleotides of DNA functions as genetic material.
3. Nucleotides are source of high energy .Ex: ATP
4. Some Nucleotides functions as co- enzymes Ex: FMN .
5. Some nucleotides functions as vitamins . Ex: FAD,NAD .

NUCLEOSIDES

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Nucleosides are composed of 2 types Nitrogen bases linked to pentose sugar. Nitrogen bases are of 2 types. They are Purines like Adenine ,Guanine and Pyrimidines like Thymine, Cytosine , Uracil.

Pentose sugar is of 2 types .they are deoxy ribose sugar that forms Deoxy ribose nucleoside and Ribose sugar that forms Ribose nucleoside .

STRUCTURE OF DNA :

Duplex model OR Double helix model OR Watson and Crick model

James D Watson and F H C Crick in **1953** with the available data provided by **wilkins** proposed the duplex of DNA. In 1962 they were awarded Noble prize. According to Duplex model DNA shows following structure :

1. DNA consists of two polynucleotide strands . These run antiparallel to each other and are twisted in right handed direction .
2. The 2 strands are made up of alternately arranged Deoxy Ribose sugar and phosphate.
3. Phosphate group is connected to third carbon atom of one sugar and fifth carbon atom of other sugar by phospho di ester bond.
4. The two strands are interconnected by cross bars made up of nitrogen bases.
5. The Nitrogen molecule is joined to the first carbon atom of the sugar by Glycosidic bond.

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6. Purines of one strand always with Pyrimidine of other strand .ie., Adenine pair with Thymine by 2 Hydrogen bonds and Guanine pair with Cytosine with 3 Hydrogen bonds .This specific base pairing is called " **Base complementality**" or " **Complementary base pairing** ".
7. The amount of Adenine is equal to the amount of Thymine and amount of Guanine is equal to the amount of cytosine .This is known as "**Chargoff's rule**".
8. The helix has diameter of 20 A .
9. DNA helix makes a full turn at every 34 A . Along this distance there is one deep major groove and one shallow minor groove.
10. Along the length of 34 A there are 10 nucleotides. The distance between each nucleotide is 3.4 A .

Alternative forms of DNA

The commonly and naturally occurring DNA described by Watson and crick is called "**B**" form DNA. This double helical structure is found to exist in other forms .They are : A & Z form DNA .

A form DNA : DNA can undergo reversible conformational changes and give rise to **A form** upon dehydration. It shows following features:

1. A form DNA is formed at 75% relative humidity, high salt ionic strength.
2. The diameter of helix is 23 A .
3. The rotation per base is 32.7 and is right handed ,it has a major groove and a minor groove.
4. 11 base pairs are present per turn .
5. The vertical rise per base is 2.56 A .

Z DNA : Z DNA was discovered by A wary and Alexander rich. It shows following features:

1. Z DNA is found in light salt concentration , can also found in solution of high ionic strength.
2. The diameter of helix is 18 A .
3. The 2 antiparallel polynucleotide chains have left handed rotation.
4. 12 base pairs are present per turn.
5. one complete turn of helix is 45 A .

DNA Replication

Replication or Duplication of DNA molecule means to make exact copies of its own structure .It takes place inside the chromosome during "S" phase of interphase.

TYPES OF DNA REPLICATION

There are 3 types of DNA replication. They are :-

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- 1)Semi conservative method
- 2)Conservative method
- 3)Dispersive method.

SEMICONSERVATIVE METHOD

This method was proposed by Watson and Crick. In 1958 it was proved by M Meselson and F W Sthal .

In Semi conservative method of DNA replication 2 daughter molecules are formed ,each with one parental strand and a new strand .

It takes place as follows :

- 1 .DNA Replication takes place at a specific point called ORI site or origin site.
2. The DNA helix unwind with the help of DNA unwinding protein , the 2 strands separate from each other after breakage of hydrogen bonds between base pairs. Single strand binding protein extend along single strand and stabilize it.
- 3 .This results in Y shaped replication fork. The strain caused by unwinding is relieved by super helix relaxing protein.
- 4 .Initiation of DNA synthesis require RNA primer .It is a short polynucleotide chain synthesized by DNA template in presence of polymerase enzyme . The separated strands act as **Templates** .

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5 .synthesis of new DNA strand takes place by addition of nucleotides to 3-oH group of RNA primer in 5- 3 direction in presence of DNA polymerase III .It take place continuously and it is referred as **Leading strand**.

6 .The other strand is synthesized in short fragments **on Lagging strand** .These are called **Okazaki fragments**. Named after the discoverer Okazaki .

8. DNA Ligase joins okazaki fragments into long polynucleotide chain.

9. **DNA polymerase I** degrades RNA primer by and simultaneously catalyses the synthesis of short DNA fragment to replace RNA primer . This segment is joined to main DNA strand by DNA ligase.

10. The newly formed daughter strands are complementary to their template and undergo coiling with it.

11.Thus 2 daughter DNA molecules are formed .Each daughter DNA has one parental strand and another new strand .Hence this method of DNA replication is called **Semiconservative method**.

12. DNA duplication is a complex process carried by multi enzyme complex called **Replisome** .The segment of DNA under replication with its origin to termination is called "**Replicon**".

Conservative method

Conservative method of DNA replication was proposed by Cavalieri and Rosenberg.

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According to this method the DNA strands do not separate but acts as templates and produce new daughter strands which wind together .

Thus 2 Daughter DNA Double helix are formed , one helix would be with newly synthesised material and the other would conserve the parental material and the conservation is 100%.

DISPERSIVE METHOD

According to this method the parental DNA molecule breaks into small fragments. Each fragment synthesise small DNA molecule. Later all the fragments become randomly connected to form two new molecule.

DNA replication in prokaryotes

In vitro DNA replication has been extensively studied in E. coli and phages .Various models have been proposed to explain the DNA replication.They are : 1) carins model. 2) Theta model .3) Rolling circle model .

ROLLING CIRCLE MODEL Rolling circle model of DNA Replication takes place in Bacteria and certain Virus

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The process of rolling circle model of DNA replication involves following steps :-

- 1 .DNA replication takes place at specific point called ORI site. Protein A recognize the site .
- 2.Nick is formed in one strand generating free 3-oH end .
- 3.Replication fork is generated by the influence of Helicase and SSB protein.
- 4.3-oH end serve as primer for the synthesis of new strand by the action of DNA polymerase .
- 5.Newly synthesized strand replaces the original strand .
- 6.Long displaced strand appear as tail joined to a circular structure . It resemble greek letter Sigma.
- 7.Displaced strand may be cut at unit length giving rise to several single stranded DNA which circularize and inserted inside phage particle.
- 8.Single strand may synthesise another strand to give rise to Duplex DNA which can be circularized . each circle replicate by Rolling circle model.

FUNCTIONS OF DNA

- 1 .DNA acts as a carrier of genetic information from generation to generation.
2. DNA is a stable macro molecule in almost all living organisms and it is immortal.
- 3 .DNA controls all biological activities of a cell .
- 4 . It consists of Genetic code for protein synthesis .
- 5 .DNA serves as a Template for synthesis of RNA.

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Ribose nucleic Acid (RNA)

- 1) RNA is a nucleic acid more than 90% of RNA is present in the cytoplasm and rest in the nucleus.
- 2) RNA is a single stranded polynucleotide produced from DNA by the process of Transcription.
- 3) Each nucleotide is composed of Ribose sugar, phosphate and Nitrogen bases.
- 4) The nitrogen bases are of two types purines like Adenine, Guanine and Pyrimidines like cytosine, uracil.
- 5) Single stranded RNA fold upon itself forming hairpin loops which show complementary base pairs.
- 6) Base composition does not follow Chargoff's rule.

Kinds of RNA

There are two types of RNA they are: 1) Genetical RNA 2) Non-Genetical RNA

- 1) **Genetical RNA**; The RNA which constitutes the genetic material is called Genetical RNA.
Ex; HIV ,TMV ,Polio myelitis, Influenza virus.
- 2) **Non-Genetical RNA** : The RNA which do not constitute the genetic material but play major role in genetic code and protein synthesis is called Non-Genetical RNA. It is present in all living organisms from bacteria to man.

These are of three types they are

- 1) Ribosomal RNA (r- RNA or Insoluble RNA)
- 2) Transfer RNA (t- RNA or Soluble RNA or Adaptor RNA)
- 3) Messenger RNA (m- RNA)

Ribosomal RNA [r RNA] or Insoluble RNA

- Ribosomal RNA is the most stable kind of Ribonucleic acid associated with ribosomes. It constitutes about 80% of the cellular RNA.
- It is a single stranded polynucleotide chain folded upon itself in some regions.
- The folded regions show base pairing. Complementary base pairs are joined by hydrogen bonds.
- The unfolded regions do not show base pairing. Thus purine and pyrimidine equality is absent.
- In Eukaryotes it is formed at nucleolar organizer region of chromosome.

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Functions of r-RNA

- 1) During protein synthesis it helps to recognize and bind m-RNA and t-RNA
- 2) It establishes peptidal binding site (P site) Amino acyl binding site (A site) in ribosome.

Transfer RNA (t-RNA) or soluble RNA

The t-RNA are single stranded polynucleotide chain made up of 70-90 nucleotides. they form 10-20 % of total RNA of the cell. They are synthesized by tRNA genes of DNA by Transcription. The first formed tRNA transcript undergo several levels of processing to become mature 3 dimensional tRNA

The structure of mature tRNA is 3 dimensional, when folded into "L" shaped molecule. It can also be in two dimensions as 'Clover leaf model'. It was proposed by Holley.

- The t-RNA is a Ribonucleic acid that transfer the activated amino acids to the ribosome to synthesise proteins.
- t-RNA is very small and remains in the supernatant during centrifugation. Hence it is also called as **Soluble RNA**
- t-RNA serve as an adaptor molecule to attach amino acids. Hence it is also called as **Adaptor RNA**.

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- t-RNA constitutes 10 to 15% of the total weight of RNA of the cell. It is single stranded with 3' and 5' ends which is folded on itself. In 1965 Holley proposed "Clover leaf model" to explain secondary structure of
-
-

t-RNA. According to this model t-RNA has 5 arms. They are :- a) Acceptor arm b) D- arm c) Anti codon arm d) Variable arm e) T arm.

The **D, T arm and Anticodon arm** shows stem and a loop. In stem there is base pairing, in loop there is no base pairing.

1. Acceptor Arm

This is 3' end of the strand with CCA unpaired base. With the help of an enzyme it recognizes the activated amino acid and binds to it by covalent bond to form Amino acyl t-RNA

2. D Arm (DHU ARM)

It contains Dihydroxy uridine nucleotide hence it is called **D arm**. In presence of t-RNA synthetase it recognizes activated amino acid.

3. Anticodon arm

It contains 5 paired bases in stem and 7 unpaired bases in loop. 3 bases of the loop form Anticodon which recognizes codon of mRNA and binds to it by hydrogen bonds.

4. Variable arm

It is a small arm with unpaired bases.

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5. T arm

It is a Ribosome identifying site and helps in binding t-RNA to it.

MESSENGER RNA (m-RNA)

Messenger RNA carries genetic information for Protein synthesis from DNA to Cytoplasm , it constitutes 3 to 5% of the total RNA of the cell . Volkin discovered it in E. coli .Jacob and Monad coined the term m-RNA .

m-RNA is an un coiled polynucleotide with 5'-3' end . It shows following structural features :-

1. **Cap** :- In Eukaryotes cap is present at 5' end . In Prokaryotes it is absent . It helps the m-RNA to bind with ribosome.
2. **Non coding region I (leader sequence)** It follows cap. It is formed of 10 – 100 nucleotides , rich in Adenine and uracil.
3. **Initiator codon** :- It follows leader sequence .AUG is the initiator codon . It initiates the processes of protein synthesis .
4. **Coding region**:- It follows initiator codon. It contains 1500 nucleotides which code for amino acids .
5. **Terminator codon** :- It follows Coding region .3 Terminator codons namely UAA ,UAG,UGA stop the process of protein synthesis .
6. **Non coding region II** :- It follows Terminator . It contains 50-150 nucleotides which never code any amino acid .
7. **Poly A sequence** :- In Eukaryotes it is present at 3' end, In Prokaryotic m-RNA it is absent . It consists of 200 to 250 Adenylate sequences.

FUNCTIONS

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1. M-RNA carries message from DNA in the form of codons to Ribosomes .
2. Single M-RNA can accommodate many ribosomes to synthesise multiple copies of same protein.

GENETIC CODE

DNA is the genetic material . The Nitrogen bases in DNA convey the message through m-RNA , to code for specific sequence of Amino acids to form proteins . This is called **Genetic code** . **OR**

The sequence of 3 nitrogen bases (Nucleotide) in m-RNA molecule which contains the information for the synthesis of protein molecule is called Genetic code .

F.H.C crick gave the idea of triplet code Marshal Nirenberg gave experimental proof for existence of genetic code. Hargobind khorana established Genetic code dictionary.

Properties of genetic code :-

A Genetic code shows following characteristics:-

1. **The code is Triplet** :- Genetic code is a Triplet code .it is represented by a sequence of 3 nitrogen bases determining an aminoacid .
2. **universal** :-genetic code is universal. A particular code determines the same amino acid in all organisms . Ex: UUU and UUC codes phenyl alanine in virus , bacteria plants and Animals .
3. **colinearity and Polarity** :-codes in polypeptide chain have linear arrangement. It has polarity. It is read in one direction 5' end to 3'.
4. **Commaless**:- There are no punctuation marks between codons .
5. **Non overlapping** :-codons are read in units of 3' nitrogen bases without overlapping.
6. **Initiator codon**:- AUG is the initiator codon . It initiates protein synthesis and codes for amino acid methionine. If AUG is absent GUG is the initiator codon.
7. **Terminator codon**:- Among 64 codons , 3 codons namely UAA,UAG,UGA terminate the synthesis of polypeptide chain. These are not read by t-RNA, but are recognized by releasing factors .these 3 codons do not code for any amino acids. Hence they are **called Nonsense codons**.

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8. Degeneracy :- An amino acid is coded by one or more codons . this is called Degenerate or Redundant codon. Ex : Alanine is coded by GCU,GCC,GCA,GCG.

9. Non-Ambiguous :-Codons are sensible and code for specific amino acid . There is no ambiguity.

WOBBLE HYPOTHESIS

The ability of t-RNA to recognize more than one codon by unusual pairing with third nitrogen base of m-RNA is called Wobble hypothesis .This is due to the non specificity of the third base of the codon.

In 1965 F.H.C Crick proposed wobble hypothesis. According to them the hydrogen bonding between anticodon and codon for first 2 bases fallow complementary pairing, but base pairing of third base of codon allow several types .This hypothesis is widely accepted because of experimental evidence.

CODE :- A sequence of 3 unpaired Nitrogen bases found in DNA.

Codon:- A sequence of 3 Nitrogen bases found in m-RNA which are complementary to codes of DNA.

Anticodon :- A sequence of 3 Nitrogen bases found in t-RNA which are complementary to codons of m-RNA .

Codes of DNA AAA ACA CGA TGC

Codons of m-RNA UUU UGC GCU ACG

Anticodons of t-RNA AAA ACA CGA UCG

Polypeptide chain Phenyl alanine-Cystine—Alanine—Serine

PROTEIN SYNTHESIS

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Proteins are macro molecules formed of 20 types of amino acids linked by peptide bonds in different combinations. It is a complex biological activity that takes place in the Ribosomes and they regulate biochemical reactions in the body.

The central dogma of protein synthesis

The unidirectional flow of genetic information from DNA to RNA to Protein is called Central dogma.

DNA by transcription form RNA. M-RNA in Ribosomewith association of t-RNA, r-RNA and enzymes by translation synthesise Proteins . This can be represented as fallows:-

DNA Transcription **RNA** Translation **Proteins.**

Mechanism of Protein synthesis

The process of protein synthesis involves 2 steps .They are :-

1. Transcription
2. Translation

TRANSCRIPTION

The synthesis of RNA on DNA is called Protein synthesis .Steps involved in Transcription are as fallows:-

M- RNA synthesis on sense strand of DNA

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1. Transcription starts at Promotor region on DNA in presence of an enzyme RNA polymerase.
2. Sigma factor of RNA polymerase recognize the Promotor site and gets attached to it .This site is called “ **Pribnow box or TATAA box** .
3. In this region DNA unwinds .one of the strand acts as template to synthesise RNA .It is called’ Sense strand ‘.The other strand is called ‘Antisense strand’.
4. After initiation of sigma factor is released from core enzyme chain elongation takes place in 5’ to 3’ direction by addition of nucleotides through RNA primer .Base pairing is complementary ie adenine pair with uracil and Guanine pair with cytosine.
5. Addition of nucleotides stops when RNA polymerase reaches particular site called “ **termination site ‘.or “Pause site”** .
6. ‘Rho factor ‘ or ‘sizing factor recognize the termination site.
7. **NUS A** (Nitrogen utilization factor –A) helps in release of new RNA molecules. DNA under go coiling.

TRANSLATION

The synthesis of polypeptide chain from Amino acids using coded information in m-RNA is called Translation. Steps involved in the process of translation are as follows ; - 1) Activation of amino acids 2) Intitiation of polypeptide chain 3) Chain elongation 4) Termination

1. Activation of amino acids

20 types of amino acids present in the cytoplasm are activated by ATP and specific amino acyl synthetase enzyme to form amino acyl adenylate.

Activated amino acid molecule bind to acceptor arm of t-RNA at CCA 3’ to form amino acyl t-RNA complex.

2 . Initiation of polypeptide chain

t-RNA molecule with n-formyl methionine binds to initiator codon

AUG on m-RNA at 30 s sub unit of ribosome in presence of

GTP(Guonsine triphosphate) and intitiation factors IF1,IF2,IF3. This is called pre initiation complex. 50 s sub unit ribosome joins with 30s sub unit to form functional 70s ribosome this is called Initiation Complex. AA1 is **at’ P’ Site** (Peptidal Site) of 70 s ribosome.

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3 . Elongation of Polypeptide Chain

t-RNA with AA2 occupy 'A' Site (Amino acyl site) peptide bond develops between AA1 and AA2, in presence of peptidyl transferase enzyme.

Methionine and t-RNA are separated by an enzyme t-RNA deacylase. T-RNA is released into cytoplasm for further aminoacylation.

Ribosome moves on codons of m-RNA. Amino acid molecules are added linked by peptide bonds thus polypeptide chain elongates in presence of EF-Ts and EF-TU.

4) Termination of polypeptide chain

When ribosome reaches terminator codons on m-RNA like UAA, UAG, UGA synthesis of polypeptide chain stops.

Releasing factors RF1 or RF2 interacts with terminator codon to form RF- Terminator codon-ribosome complex which blocks further elongation.

2 subunits of ribosome dissociate, polypeptide chain is released as a specific protein.

POLY RIBOSOME

Single m-RNA with many Ribosomes to synthesise multiple copies of same protein is called Poly ribosome.

After ribosome has translated about 25 codons the 5' end of m-RNA is free. The second ribosome forms initiation complex, moves along m-RNA to produce second polypeptide chain. It is followed by third, fourth, fifth and so on. Thus series of Ribosomes on m-RNA are involved in synthesis of same type of protein.

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GENE REGULATION

F. Jacob and Monod in 1961 proposed Operon model of gene regulation in Prokaryotes. Ex: E. coli. They were awarded noble prize .

Operon is a unit of bacterial gene expression and regulation . It consists of Structural genes ,Control genes ,Regulator genes and effector molecule.

1.Structural genes :- Structural genes include Cistrons that codes for m-RNA to produce specific enzymes .inLac operon it include Z,Y,A genes. Z gene transcribe for B-Galactosidase enzyme , enzyme ,Y gene for permease ,A gene for transacetylase enzyme.

Z gene transcribe m-RNA for B-galactosidase enzyme ,Y gene for Permease enzyme and A gene for transacetylase enzyme .

2.Controlling elements :- Controlling genes include promotor and Operator genes . These regulates function of structural genes .

Promotor gene is a specific gene sequence in DNA, recognized by RNA polymerase and initiate Transcription .

Operator gene is a specific base sequence in DNA present between promotor and structural gene. It interact with regulatornprotein to promote or prevent transcription.

3.Regulator protein :- Regulator gene produce a protein called Repressor Protein(Regulator protein).It has potentiality to specifically bind to controlling elements due to their 3 dimensional structure.they may be linked or unlinked to structural genes.

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4.Effector molecules

Effector molecules are called Inducer or co repressor .These may be sugar ,Amino acids that bind to regulator protein.

In prokaryotes operon is of 2 types , namely

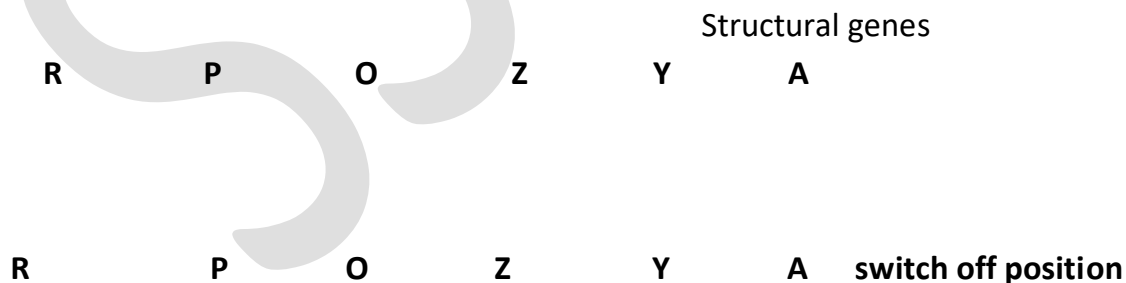
- a) Inducible operon,
- b) Repressible operon.

A) Inducible operon

Inducible operon functions in presence of inducer like Lactose . Hence it is **called Lac operon**.

In absence of Inducer Lactose in the cell, the regulator gene produce repressor protein . It binds to operator gene ,then switch is off .This is called Repressed stage The Promotor gene prevents transcription of m-RNA . Thus enzymes are not produced for Lactose utilisation.

In presence of lactose in the cell , lactose binds to repressor protein produced by regulator gene , switch is on . This is called derepressed stage. The promotor gene promotes transcription of m-RNA by structural genes . Thus enzymes essential for lactose utilization are produced.



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R P O Z Y A switch on position

R P O Z Y A Transcription of
m-RNA
B- galact permease
oxidase

GENE REGULATION IN EUKARYOTES

Gene regulation in Eukaryotes is explained by following hypothesis:-

1) RNA depressor model:-This model was proposed by frenster . Hence it is also called Frenster.

According to this model histone and non-histone proteins associated with DNA of chromosomes play role in gene regulation. Histones acts as Repressor protein bring about repression of transcription .

Nuclear polyions form complexes with histones , separate it from DNA and initiate transcription of m-RNA for protein.

2) Non- Histone derepressor model

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This model was proposed by Paul and co worker .According to this model histone bring about non specific repression of transcription . gene specific non histone binds with histone , remove it from DNA. The 2 strands of DNA seperates one of the strand transcribe m-RNA for protein synthesis.

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VITAMINS

Vitamins are special organic compounds supplemented to the body by external nutrition, these constitute an integral and essential part of balanced diet . They are required in small quantities and are essential for normal functioning of the body. Their deficiency leads to abnormality both in structure and physiology. Excess of vitamins also causes abnormality known as Hypervitaminosis with toxic effects.

Classification : vitamins are classified into 2 types. They are :

Fat soluble Vitamins : These are associated with fats of natural food , and absorbed along with dietary fat ..Ex : vitamin A ,K, D ,E. I]

water soluble vitamins: These are integral part of daily diet. Ex: Vitamin C, B complex. II]

I] Fat soluble Vitamins

1] Vitamin A (Retinol) :

Egg, Butter, Milk, carrot ,cod liver oil ,shark liver oil.

source –

Functions : 1)

Maintain the integrity of epithelium .**2)** It helps to repair and maintain tissues. **3)** It helps in resistance to infection **4)** It is necessary for formation of Retinal pigments like Rhodopsin Iodopsin .

Effects 1) Deficiency of Vitamin A causes Night blindness (Xerophthalmia) . **2)**

Hypervitamins have toxic effects and leads to loss of appetite, weight loss ,bleeding and cracking of lips, hair loss. In severe cases liver enlargement and pain in bones and joints.

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2] Vitamin D (Calciferol)

Source

: Eggs and fish oils but not in vegetable oils. In human it is synthesized in skin from derivative of cholesterol under the influence of ultraviolet rays of sunlight. Hence it is also considered as hormone.

Functions ; 1] It regulates the absorption and utilization of Phosphorus , calcium.

2] It is

important in blood clotting and nerve cell maintenance.

Effects 1] Deficiency

results in bone disease called Rickets in children and osteomalacia in adults.**2]** Hypervitaminosis results in weight loss, nausea ,polyuria, induce renal stone ,calciferation of arteries.

3] Vitamin E (Tocopherol)

Source : Vegetable oil,green vegetables, milk, eggs , butter, wheat germ oil, Cotton seed oil, Soya bean oil and Palm oil.

Functions :1] It inhibits

peroxide formation that prevents damage to membrane lipids.**2]** It maintain structural integrity of membrane.

Effects : 1] Deficiency brings decrease in quantity

of unsaturated fats in the cell

2] Abnormality in structure and functions of organells like

Mitochondria , Lysosomes, cell membrane. **3]** Induce sterility and degeneration of muscles.**4]** increase fragility of erythrocytes.

4]Vitamin K (Phylloquinone)

Source : dark vegetables like Spinach, Cauli flower, peas, cabbage and Cereals.molasses, liver, Yoghurt, Safflower oil.In human body it is produced by intestinal micro organisms.

Function ; It is important in

blood clotting.

Effects : Deficiency of

Vitamin K results in delayed coagulation of blood .**Hypervitaminosis** brings haemolytic anaemia.

2] Water soluble Vitamins: Water soluble vitamins are parts of daily diet and parts of enzymes as coenzymes. Ex: Vitamin C and groups of B complex.

Vitamin

C

[

Ascorbic

acid]

source : Citrus, Gooseberry, Leafy vegetables, Tomatoes, milk, Liver.

Functions : 1] It plays major role in cellular respiraton.**2]** It help in absorption and utilization of iron, metabolism of Carotene, Steroids. **3]** It plays significant role in Tyrosine metabolism.**4]** It helps in wound healing.

Effects: 1] Deficiency of

vitamin C causes swelling and bleeding of Gums ,connective tissue abnormalities which leads to Scurvy .**2]** hypervitaminosis brings about Diarrhoea, flatulens, uricosuria, pancreatic damage and impairment of leucocyte bactericidal activity .

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Vitamin B Complex It include major vitamins like Thiamine(B) , Riboflavin (B) , Patathonic acid (B) , Nicotinic acid (Niacin,B) Pyridoxine (B) Folic acid (B) Biotin , Cobalamin (B , cyanocobalamin)

Source : yeast, Liver, Rice polishings .

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GENETIC ENGINEERING

The alteration of genotype of an organism using invitro techniques to produce products and services of human benefit is called ' **Genetic engineering** ' .

In 1973 Stanly cohen (Stanford university , USA) hebert bayer (University of California USA) reported that desired gene can be isolated and joined into the plasmid of another organism using enzymes.

Tools used in genetic engineering : The biological tools used in genetic engineering are : Vector , Enzymes ,desired gene ,Host cell, Bio reactor.

1] Vector : Vectors are DNA molecules that can carry a foreign DNA fragment inserted into them without any disturbance in their expression .. It is also called as 'Cloning vehicle ' or 'Vehicle DNAs'. It may be Plasmid ,Bacteriophage ,transpos. A vector must posses the following charecteristics :-

1. Vector must replicate in host cell after its introduction.
2. A unique cleavage site must be present in one of the marker gene.
3. It must posses marker gene such as Resistance for Tetracycline , kanamycin , Ampicillin.
4. It should contain specific control systems like Promoters, Terminators ,Ribosome binding site.

2] Plasmid Plasmids are extrachromosomal ,self replicating ,Double stranded , circular DNA molecules present in Bacterial cell. In 1973 Cohen et al.,reported cloning DNA using Plasmid as Vector . Plasmids are used as cloning vehicles when they posses following features :-

1. plasmid can be readily isolated from the cells.
2. It posses single restriction site for one or more restriction enzymes .
3. Insertion of a linear molecule at one of these sites does not alter its replication properties.
4. It can be reintroduced into a bacterial cell and show normal activity in the parent cell.
5. They do not occur free in nature but are found in bacterial cells .

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TUMKUR UNIVERSITY PRACTICAL EXAMINATION

II SEMESTER PRACTICAL QUESTION PAPER

TIME : 3 Hrs **BOTANY PAPER -- II** **MAX MARKS : 30**

- 1] Identify the specimens **A , B ,C** giving reasons . [Identification , classification = 1 mark ,Labeled diagram and reasons =2 marks] **3x3= 9**
- 2] prepare a temporary safranin stained T S of the material **D** sketch, label ,identify with reasons .leave the preparation for evaluation . [Staining, mounting=3 marks, labeled diagram = 1 mark, identification and reasons =1 mark] **1x5 = 5**
- 3] Write critical notes on **E** [Identification =1 mark, Notes = 1 mark] **1x2=2**
- 4] Identify the slides **F,G,H** with labeled diagrams and description. [Identification = 1mark,Labeled diagram =1 mark, reasons =1 mark] **3 x3 =9**
- 5] Class records and submissions . **3+2 =5**

TUMKUR UNIVERSITY PRACTICAL EXAMINATION

II SEMESTER PRACTICAL QUESTION PAPER

TIME : 3 Hrs **BOTANY PAPER -- II** **MAX MARKS : 30**

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- 4]** Identify the slides **F,G,H** with labeled diagrams and description. [Identification = 1mark,Labeled diagram =1 mark, reasons =1 mark] **3 x3 =9**
- 5]** Class records and submissions . **3+2 =5**

TUMKUR UNIVERSITY PRACTICAL EXAMINATION

IV SEMESTER PRACTICAL QUESTION PAPER

TIME : 3 Hrs

BOTANY PAPER -- IV

MAX MARKS : 30

- 1]** Identify and classify specimens **A and B** giving reasons . [identification , classification =1 mark, reasons= 2 marks] **3x2= 6**
- 2]** Identify the slides **C, D and E** with reasons and labeled diagrams. [identification=1mark,reasons=1m,diagram & labeling=1 mark] **3x3=9**
- 3]** Mount the Endosperm / Embryo of specimen **F** and Comment. [mounting =4 marks, comment ,diagram = 2 marks] **4x2=6**
- 4]** Comment on **G** .[Diagram=1 mark, comment=3 marks] **4x1=4**
- 5]** Class records and permanent slides submissions . **3+2 =5**

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TUMKUR UNIVERSITY PRACTICAL EXAMINATION

IV SEMESTER PRACTICAL QUESTION PAPER

TIME : 3 Hrs

BOTANY PAPER -- IV

MAX MARKS : 30

- 1]** Identify and classify specimens **A and B** giving reasons . [identification , classification =1 mark, reasons= 2 marks] 3x2= 6
- 2]** Identify the slides **C, D and E** with reasons and labeled diagrams. [identification=1mark,reasons=1m,diagram & labeling=1 mark] 3x3=9
- 3]** Mount the Endosperm / Embryo of specimen **F** and Comment. [mounting =3 marks, comment ,diagram = 2 marks] 6x1=6
- 4]** Comment on **G** .[Diagram=1 mark, comment=2 mark] 3x1=3
- 5]** Class records and permanent slides submissions . 3+2 =5

TUMKUR UNIVERSITY PRACTICAL EXAMINATION

VI SEMESTER PRACTICAL QUESTION PAPER

TIME : 3 Hrs

BOTANY PAPER -- VII

MAX MARKS : 30

- 1]** Conduct the biochemical test of the sample **A** . [Positive test =2 marks, Negative test = 4 marks] 06
- 2]** Determine the osmotic potential of cell sap by Plasmolytic method/Stomatal index of material **B** [conducting the experiment =2 marks, principle=2 marks, procedure=2 marks ,result = 2 marks] 08

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- 3]** Determine the pH of the given sample **C**. .[Determination of
pH=1 mark, comment =2 mark] 03
- 4]** Comment on experiments **D and E** . [
Identification =1 mark, Comment = 3 marks] 03
- 5] Class record** 05

TUMKUR UNIVERSITY PRACTICAL EXAMINATION

VI SEMESTER PRACTICAL QUESTION PAPER

TIME : 3 Hrs

BOTANY PAPER -- VII

MAX MARKS : 30

- 1]** Conduct the biochemical test of the sample **A** .
[Positive test =2 marks, Negative test = 4 marks] 06
- 2]** Determine the osmotic potential of cell sap by Plasmolytic method/Stomatal index of material **B** [conducting the experiment =2 marks, principle=2 marks, procedure=2 marks ,result = 2 marks]
08
- 3]** Determine the pH of the given sample **C**. .[Determination of
pH=1 mark, comment =2 mark] 03
- 4]** Comment on experiments **D and E** . [
Identification =1 mark, Comment = 3 marks] 03
- 5] Class record** 05

TUMKUR UNIVERSITY PRACTICAL EXAMINATION

VI SEMESTER PRACTICAL QUESTION PAPER

III B.Sc VI SEMESTER BOTANY PAPER-VII
Plant physiology and Metabolism

TIME : 3 Hrs

BOTANY PAPER -- VIII

MAX MARKS : 30

- 1]** Separate the photosynthetic pigments from the sample **A** by paper chromatography and measure their R f values. [Requirements- mark , Principle – procedure - mark , Conduction of expt- mark , Result & calculation- mark.] **10**
- 2]** Estimate the Ascorbic acid content in the sample **B** . [Requirements- , Principle – procedure - , Conduction of expt- , Result & calculation] **07**
- 3]** Comment on Experiments **C and D**. [Idetification of expt- mark , Principle- , Comment- mark] **2x4 =08**
- 4]** **Class record**
- 05**

TUMKUR UNIVERSITY PRACTICAL EXAMINATION

VI SEMESTER PRACTICAL QUESTION PAPER

TIME : 3 Hrs

BOTANY PAPER -- VIII

MAX MARKS : 30

- 1]** Separate the photosynthetic pigments from the sample **A** by paper chromatography and measure their R f values. [Requirements- mark , Principle – procedure - mark , Conduction of expt- mark, Result & calculation- mark .] **10**
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- 3]** Comment on Experiments **C and D**. [Idetification of expt- mark , Principle- mark , Comment- mark.] **2x4=08**
- 4]** **Class record**
- 05**

TUMKUR UNIVERSITY PRACTICAL EXAMINATION

VI SEMESTER PRACTICAL QUESTION PAPER

TIME : 3 Hrs

BOTANY PAPER -- VIII

MAX MARKS : 30

- 1]** Separate the photosynthetic pigments from the sample **A** by paper chromatography and measure their R f values. [Requirements- mark , Principle – procedure - mark , Conduction of expt- mark, Result & calculation- mark .] **10**
- 2]** Estimate the Ascorbic acid content in the sample **B** . [Requirements- mark , Principle – procedure - mark ,

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Plant physiology and Metabolism

Conduction of expt- mark , Result & calculation] **07**

Experiments **C and D.**

Principle- , Comment- mark .]

05

3] Comment on

[Identification of expt- mark ,

2x4=08

4] Class record

TUMKUR UNIVERSITY PRACTICAL EXAMINATION

BATCH B

VI SEMESTER BOTANY

TIME: 10=1pm

PRACTICAL PAPER VII

DATE: 08-04-2013

SL.NO REGISTER NUMBER ANSWER BOOK NO. SIGNATURE

TUMKUR UNIVERSITY PRACTICAL EXAMINATION

BATCH B

VI SEMESTER BOTANY

TIME: 2-5 pm

PRACTICAL PAPER VII

DATE: 08-04-2013

SL.NO REGISTER NUMBER ANSWER BOOK NO. SIGNATURE

TUMKUR UNIVERSITY PRACTICAL EXAMINATION

BATCH B

VI SEMESTER BOTANY

TIME: 10-1 pm

PRACTICAL PAPER VII

DATE: 09-04-2013

SL.NO REGISTER NUMBER ANSWER BOOK NO. SIGNATURE

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TUMKUR UNIVERSITY PRACTICAL EXAMINATION

BATCH B

VI SEMESTER BOTANY

TIME: 2-5 pm

PRACTICAL PAPER VII

DATE:10-04-2013

SL.NO	REGISTER NUMBER	ANSWER BOOK NO.	SIGNATURE
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2] Internal

Total no of Students assigned =

No of students attended=

No of Absentees =

Examiners 1]External

2] Internal

Total no of Students assigned =

No of students Attended =

No of Absentees =Examiners 1]External

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Plant physiology and Metabolism

2] Internal

Total no of Students assigned =

No of students Attended =

No of Absentees =

Examiners 1]External

2]Internal

Total no of Students assigned =

No of students Attended =

No of Absentees =

Examiners 1]External

2]Internal

II	<u>SEM</u>	<u>Specimens</u>	[2 fungi	+	1 Bryophytes]
A							<u>B</u>
<u>C</u>							
D						–Micropreparation	
E---Pathology						<u>Slides</u>	[1
fungi	,	1		Bryophytes	,	1	Anatomy]
F							G
H							

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II SEM Specimens [2 fungi + 1 Bryophytes]
 A B
 C
 D –Micropreparation
 E---Pathology Slides [1
 fungi , 1 Bryophytes , 1 Anatomy]
 F G
 H

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 A B
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III B.Sc VI SEMESTER BOTANY PAPER-VII
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fungi , 1 Bryophytes , 1 Anatomy]
F G
H

II SEM Specimens [2 fungi + 1 Bryophytes]

A B

C

D -Micropreparation

E---Pathology Slides [1

fungi , 1 Bryophytes , 1 Anatomy]

F G

H

IV SEM Specimens Gymnosperms

A B

Slides

Gymnosperms,microsporogenesis , megasporogenesis

C D

E F --- Endosperm/Embryo

G ---Pollen germination, pollinia, pollen grains

IV SEM Specimens Gymnosperms

A B

Slides

Gymnosperms,microsporogenesis , megasporogenesis

III B.Sc VI SEMESTER BOTANY PAPER-VII
Plant physiology and Metabolism

C D
E F --- Endosperm/Embryo
G ---Pollen germination, pollinia, pollen grains

IV SEM Specimens Gymnosperms
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Gymnosperms,microsporogenesis , megasporogenesis

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Plant physiology and Metabolism

VI Semester B.Sc. Examination , May/June 2013 Botany Paper – VII

[Molecular Biology ,Genetic engineering, Biotechnology , plant physiology -1]

Scheme of valuation - Question paper code - 22637

K S Gitanjali SSCASC

A . Answer any Six of the following:

6x 2 = 12

1) Mention any 4 characteristics of RNA.

½ mark each.

RNA is a nucleic acid 90% is present in cytoplasm & rest in nucleus, single stranded polynucleotide composed of Ribose sugar, Phosphate, Nitrogen bases ,Nitrogen bases are purines like Adenine, Guanine, and pyrimidines like Cytosine Uracil, Single strand is folded upon itself to form hair pin loops which show complementary base pairing, Base composition does not follow Chargoff's rule, It can't self replicate but synthesized from DNA .

2)

acid it codes .

1+ 1

Define initiator codon. Name the amino

The codon AUG that begins the protein synthesis is called Initiator codon. In Eukaryotes it codes for Methionine, In Prokaryotes it codes for N- Formyl methionine. Less frequently GUG is initiator codon & it codes for Valine.

3)

Mention 4 applications of genetic engineering in horticulture. ½ each . Transgenic plants have been engineered by Scientists with special beneficial properties like A) Value addition to crops- Potato rich in starch, Soya bean that yields Cocoa oil, Tomato that ripen slowly etc, B) Herbicide ,Virus, Disease resistant plants-Tomato resistant to fruit worm ,Papaya resistant to Ring spot virus etc, C) Ability to fix Nitrogen by non leguminous plants , D) To alter flower quality, colours in Petunia Rose, Lotus, Chrysanthimum etc.

4)

example .

1+1

What are Molecular scissors ?Give an

An Enzyme that recognise specific Palindromic sequence in DNA on both the strands and cut it with in the same recognition are called Molecular Scissors or Restriction endonuclease .EX : Eco RI , Hind II ,Hind III, Hpa II, Hae II,Taq I ,Hpa I Etc

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- 5) **Differentiate between Plasmolysis and Deplasmolysis.** **1+1** **Plasmolysis** – Shrinkage of Protoplast due to Exosmosis when cell is present in hypertonic solution and cell become flaccid. **Deplasmolysis** – Regain of normal condition of protoplast in plasmolysed cell due to Endosmosis when cell is present in hypotonic solution and cell become Turgid.
- 6) **What is Vein loading and Unloading ?** **1+1** **Vein loading** –The movement of solutes from sites of photosynthesis into the Sieve elements of Phloem in vein. **Vein unloading** –The movement of solutes from Sieve elements of phloem into the receiver cells for consumption is called Vein unloading.
- 7) **Differentiate between Transpiration and Guttation** **1+1** **Transpiration** :- Loss of water in vapors form through Stomata, Cuticle or Lenticel during day time .Transpired water is pure, gives cooling effect in plants . **Guttation**:- Loss of water in liquid form through Hydathode during night or early morning. Guttated water contain minerals, organic & inorganic substances , does not give cooling effect
- 8) **Ion Antagonism** . **Definition 1+ Example- 1** Neutralizing the toxic effect of particular element by maintaining ionic balance is called Ion Antagonism .Ex : For barely plant manganese concentration of 300-400 ppm is toxic, but it is neutralized when Silicon is present in the medium .

B. Answer any Six of the following :

6x4 =24

- 9) Griffth 's experiment :** **1+1+1+1** **1)** Fredrich griffth , a British medical officer(Bacteriologist) while working on pathogenicity of streptococcus pneumonia(it Cause pneumonia in mammals) observed in two strains of bacteria. They are :a)**Virulent smooth strain(S)** : It has polysaccharide capsule. It produce smooth colonies in culture. b) **Avirulent Rough strains(R)** : It lacks polysaccharide capsule.it produce rough colonies in culture .
- 2)** Griffth showed that heat killed' S' strain when injected into experimental mice did not cause pneumonia . **3)** When such heat killed strain were injected to mice along with living' R' strain they died of pneumonia.
- 4)** Autopsis of mice showed presence of' S' strain instead of living R strain . According to Griffth some

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chemical present in 'S' strain is responsible for change of 'R' strain into 'S' strain he called it as "Transforming principle .

10) Explain Transcription Labeled diagram- 1 ½ , Definition - ½ , Explanation -2 Synthesis of RNA from DNA is called Transcription . **1)** It starts at promoter site on DNA, it is recognized by sigma factor of RNA polymerase and attaches to it . it is called Pribnow Box or TATAA Box **2)** DNA unwinds , Sense strand transcribes m-RNA . After initiation sigma factor is released from core enzyme **3)** chain elongation takes place 5'to 3' end by addition of nucleotides, A pair with U,T pair with A and G with C. **4)** Termination site is recognized by Rho factor , addition of nucleotides stops, NUS-A release new mRNA , DNA under goes coiling.

11) Hazards of Genetic engineering . 8 hazards ½ each

1)Accidental escape of transformed microbes from laboratory into environment will pose great damage .**2)**This technique can be misused and unleash biological war fare on enemy countries .Ex: Air or water bodies might be sprayed with pathogenic microbes like plague, Anthrax etc.**3)** Genetically modified product may be toxic and threat to human health. Ex: L-tryptophan caused eosinophilia myalgia syndrome. **4)** Genetically engineered weed variety of crop become super weed . **5)** Possibility of revival of fossil organisms and use of fossil DNA. **6)** Genetic engineering patenting like terminator technology results in infertile seeds leading farmers for economic crisis. **7)** Leads to global monopoly and affect Bio diversity.**8)** Genetic engineering research tinkers the basis of evolutionary process and disturb the fine tuned balance and relationship that is existing between organisms

12) Water potential and its components . Definition -1, Components-1+1+1.

Chemical potential of water is called water potential. In solvent system it represents difference between the chemical potential of water in that system and pure water .It is expressed by megapascals or bars and denoted by letter psi (ψ). water potential of pure water is zero .

In a plant water potential is sum total of 3 components .namely matric, solute and pressure potential.

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- a) **Matric potential ()** : matric potential is defined as the amount by which water potential of the cell sap is reduced due to adsorption of water molecules by hydrophilic colloids of protoplasm . It is not significant as it does not allow free movement of water molecules.
- b) **Solute potential ()** : Solute potential is defined as the amount by which water potential of cell sap is reduced due to solute particles present in it. It represent negative numbers.
- c) **Pressure potential ()** : Pressure potential is defined as the amount by which water potential of the cell sap is reduced due to wall pressure (Pressure exerted by cell wall on cell contents) and Turgor pressure (equal pressure exerted by cell membrane on cell wall) . It represents positive sign.

13) Explain steps involved in penicillin production . **1+1+1+1**

Penicillin is an Antibiotic extracted from penicillium notatum by Alexander Fleming. The yield of penicillin from p.notatum is very low and it could be easily destroyed by acid and heat. Hence now a days Penicillium chrysogenum is used for large scale production of penicillin by following steps:

- a) **Selection of strain** : High yielding varieties of p. chrysogenum are selected. They are genetically instable ,hence maintained and stored carefully in frozen state in liquid nitrogen or fine suspension of spore is mixed with inert material like soil or sand and kept under desiccation.
- b) **Preparation of inoculum** : Pure inoculum of p.chrysogenum is developed to initiate fermentation using moyer & Corgill culture medium ,Nitrogen source is ammonium acetate, Ammonium sulphate , corn steep liquor supply potassium, dihydrogen phosphate, Magnesium sulphate.
- c) **Incubation** ; production tank is incubated using pressure to push 10% of inoculum into fermentation tank at 25- 26 c for 3-5 days. Periodic survey is done to check contamination if any and to determine quantity.
- d) **Harvest and recovery** : aseptically remove mycelium using Rotatory vacuum filter. extract penicillium using counter current solvent extraction .Adjust PH of filtrate to acidic state (2.5)using sulphuric acid.it is back extracted into an alkaline buffer with PH 7- 7.5 .Thus crude penicillin is obtained .It is further treated with aqueous sodium hydroxide followed by charcoal to eliminate pyrogens and then filtered by Seitz filter to eliminate bacteria. For medicinal purpose dry powder is stored in vials.

14)_ Significance of osmosis. **8 uses** **½ mark each**

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Plant physiology and Metabolism

1. Osmosis helps in Absorption of water 2) Movement of water between cells in the plant body 3) Maintain shape, size posture, stature due to turgidity, 4) Seismonastic movement in mimosa pudica 5) It is responsible for Stomatal movement 6) Dehiscence of Sporangia & bursting of fruit 7) Develops resistance to drought, frost 8) Turgid cells of root tip easily penetrate in soil particles 9) Plasmolysis prevent the growth of moulds & bacteria in preserved food stuffs like pickles, jams.

15) Explain starch sugar interconversion hypothesis. Stomata open-2, Close -1, obj-1

Starch Sugar inter conversion theory was proposed by Sayre in 1926, later modified by Steward in 1964. pH of the medium decides the inter conversion of starch and sugar results in stomatal movement. **During Day** time carbon dioxide liberated by respiration is used by mesophyll for photosynthesis, pH increases, insoluble starch is converted into Glucose-1-phosphate by phosphorylase, it is converted into Glucose-6-phosphate by phosphoglucomutase which is further converted into soluble Glucose and phosphate. concentration of cell sap increases, osmotic pressure increases, water diffuses into guard cell from subsidiary cells, Guard cell become turgid and stomata opens. **During night** carbon dioxide accumulates, form carbonic acid, pH decreases, Glucose is converted into Glucose-1-phosphate using ATP in presence of Hexokinase. It is converted into starch in presence of phosphorylase. Concentration of cell sap decreases, water diffuses out of guard cell, turgor pressure decreases, guard cell become flaccid, stomata close. **Objections** 1) Monocots do not have starch 2) No evidence to show presence of sugar when starch disappears 3) Stomata close at mid day without change in starch conversion. 4) Stomatal movement do not require any energy.

16) Protoplasmic streaming hypothesis. Diagram -1, Explain-2, objections-1

This theory was proposed by Dutch Botanist Hugo de Vries, supported by O. F. Curtis. According to them, solute translocation is due to combination of Diffusion and protoplasmic streaming. Organic solutes translocate by protoplasmic streaming, when it reaches end of sieve tube migrates to the next sieve tube through sieve pores by diffusion. It explains bidirectional movement of metabolites. **Objections** : 1) According to Esau there is no cyclosis in sieve tube, even it exists in young decreases with maturity. 2) Phloem exudate is from vacuole and not from cytoplasm indicating solutes are transported through the vacuoles of sieve tubes.

C. Answer any Three of the following :

3x8= 24

17) Explain semi conservative method of DNA replication.

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Definition-1, labelled diagram- 3 , Explanation - 5

To make exact copies of its own structure is called **Replication or Duplication** .It takes place inside the chromosome during “S” phase of interphase. In DNA, Replication is by Semi conservative method where **2 daughter DNA molecules are formed each with one parental strand and a new strand** .It was proposed by Watson and Crick In 1958 & proved by M Meselson and F W Sthal . It takes place as follows :-

1)DNA Replication takes place at a specific point called ORI site or origin site.**2)** The DNA helix unwind with the help of DNA unwinding protein , the 2 strands separate from each other after breakage of hydrogen bonds between base pairs. Single strand binding protein extend along single strand and stabilize it. **3)**This results in Y shaped replication fork. The strain caused by unwinding is relieved by super helix relaxing protein. **4)**Initiation of DNA synthesis require RNA primer .It is a short polynucleotide chain synthesized by DNA template in presence of polymerase enzyme . The separated strands act as **Templates** . **5)**Synthesis of new DNA strand takes place by addition of nucleotides to 3-OH group of RNA primer in 5'- 3' direction in presence of DNA polymerase III .It take place continuously and it is referred as **Leading strand**.**6)**The other strand is synthesized in short fragments on **Lagging strand** .These are called **Okazaki fragments**. Named after discoverer Okazaki **7)** DNA Ligase joins okazaki fragments into long polynucleotide chain.**8)** DNA polymerase I degrades RNA primer by and simultaneously catalyses' the synthesis of short DNA fragment to replace RNA primer . This segment is joined to main DNA strand by DNA ligase.**9)** The newly formed daughter strands are complementary to their template and undergo coiling with it.**10)**Thus 2 daughter DNA molecules are formed .Each daughter DNA has one parental strand and another new strand .Hence this method of DNA replication is called **Semiconservative method**.**11)** DNA duplication is a complex process carried by multi enzyme complex called **Replisome** .The segment of DNA under replication with its origin to termination is called “**Replicon**”.

18) Describe physical force theories of Ascent of sap. Capillary force, imbibition, Atmospheric theory – 1 each (3) Transpiration pull - Fig-2, Expla-2, object- 1 (5)

According to this theory physical forces in xylem elements of plants are responsible for Ascent of sap.

1) Capillary force theory -According to Boehm capillary force in xylem vessel is responsible.

Objection:- Capillary force can not function due to cross wall at each cell, magnitude is low, ends of vessels are not dipped in water, lower terrestrial plants have only tracheid's. **2) Imbibition theory** :-

According to Sachs & Unger Imbibitional force is responsible for Ascent of sap. Objection:- Ascent of

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Plant physiology and Metabolism

sap is through lumen of vessel and not through wall. **3) Atmospheric theory** :- According to this theory water moves up in xylem to fill up drop in atmospheric pressure due to loss of water during transpiration. Objection:- No vacuum at upper end of plant for atmospheric pressure to elevate water beyond 30ft., lower end of the column do not directly open in soil water

4) Transpiration pull theory :- This theory was proposed by Dixon & Jolley, supported by Renner, Curtis, Clark, Levitt. According to this theory two forces are responsible for ascent of sap. They are **a)** Cohesive & Adhesive properties of water to form water column. **b)** Transpiration pull exerted on this column. Water molecules are held together tightly due to strong cohesive force (mutual force of attraction due to hydrogen bonds between them) They also have Adhesive property i.e. strong attraction between water column and inner walls of xylem. Thus continuous water column is formed from leaf to root which can not be broken. The water net has two terminals. Root tip near absorbing region and sub-stomatal cavity in mesophyll. Transpiration creates DPD, resulting in flow of water from adjacent mesophyll cells, this DPD reaches cells abutting vasculature & xylem elements. Due to continuous transpiration, high DPD causes tension on the water column & it is transported down into root up to area of absorption. Thus water is pulled up due to suction force (Transpiration pull) due to transpiration. Objection: - Entry of air bubbles in the xylem disturbs the continuity of water column.

19) Explain Active absorption of Water . add a note on factors affecting absorption.

Osmotic absorption -2, non osmotic absorption -2, any 4 factors - 4

Absorption of water due to force generated in root cells is called active absorption. It takes place by 2 methods. They are :- 1) Osmotic absorption. 2) Non osmotic absorption.

1) Osmotic absorption: -Atkins & Priestly proposed it. Absorption of water due to concentration gradient between cell sap & soil solution is called osmotic absorption. Water potential of cell sap has higher negative value than the soil solution, hence osmotic migration of the solvent takes place into the cell. Obj:- Cell sap conc. is not always high, Root pressure is not universal in plants.

2) Non osmotic absorption :- It was proposed by Clark & supported by Thiamann, Bogen. Absorption of water against concentration gradient utilizing metabolic energy is called Non osmotic absorption. The evidences that support it are as follows:- 1) Respiratory inhibitors like Malonate decrease water absorption. 2) Poison like KCN which retard metabolic activities of root cells retard water absorption. 3) Growth hormones that increase metabolic activities of cells stimulate absorption. 4) low temp. that retards rate of energy release during respiration reduces water absorption.

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Factors affecting absorption :-**External factors** are **1)soil water**-its increase beyond limit results in poor aeration, retard metabolic activities of root cell, retard absorption.**2) Soil solution** :-increase concentration of soil solution increase osmotic pressure than cell sap and suppress absorption.**3)soil air** ;- poor aeration accumulate carbon dioxide released by root cells during respiration, increases viscosity of protoplasm and reduces permeability.**4) Soil temp**:- Vey high soil temp .kills living tissue of root &retard absorption low temp. increases viscosity of protoplasm & become less permeable to water .**Internal factors:- 1) Transpiration**- high rate of transpiration increases absorption due to adhesion of water molecules.**2) Root system** ; extensively branched root system, root hair per unit area increase s absorbing regions.**3) metabolism**:-Poor metabolic activities reduces available energy for all physiological activities ,reduces absorption.

20) Give an account on Recombinant DNA technology .
Schematic representation – 2, 4 steps - 1½ each step (6 marks)

The technique of isolation of desired gene and its transplantation into the plasmid of an organism and activate it to produce desired product is called Recombinant DNA technology. It involves 4 steps :- 1) Isolation of desired gene from donar.2) Insertion of desired gene into plasmid.3) Transfer of r plasmid into host .4) Culture of transformed cells to synthesize desired product .

1) Isolation of desired gene from donor :- Isolation of desired gene from DNA of an organism is done by **a) Shot gun method** :-In this method using specific restriction enzymes the isolated DNA from a particular organism is cleaved into many fragments .**b) Reverse transcription** :-In this method,mRNA segments of desired gene are used are used as template to produce fragment of single stranded DNA in presence of reverse transcriptase .This DNA is complimentary to mRNA ,hence it is called cDNA .hydrolysis using alkaline sucrose cDNA separates from mRNA. Single

stranded cDNA acts as template is converted into double stranded DNA in presence of DNA polymerase I .SI nuclease breaks the covalent linkage between two DNA strands .

2) Insertion of desired gene into Plasmid :-Plasmid is an extra chromosomal, self-replicating, double stranded circular DNA present in some Bacteria. It is used as vector for cloning desired gene .Desired gene is inserted into Plasmid as fallows :- **a)** Plasmid is cut with restriction endonuclease , it produces DNA with sticky ends .**b)**Plasmid and desired gene are mixed together. Sticky ends of plasmid and desired gene link by complementary base pairing. **c)**Ligase seal the nick found between them . thus recombinant DNA is obtained

3)Transfer of r DNA into Host cell:- The E.coli , is a favorite cloning organisms as they do not live in human beings and are nonpathogenic. There are several methods to transfer r DNA into host cell. It depends on type

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of vector & host cell. In Transformation E. coli are pretreated with cacl at low temp. and rDNA is mixed with it. rDNA migrate into it . **4)**

Culture of Transformed cells :- Host cell with recombinant DNA are identified and separated. It is called Screening or Selection. Transformed cells are grown in suitable nutrient medium in bioreactors. If Eukaryotic gene is inserted into Prokaryotic organism, as protein synthesizing machinery of prokaryote is different from eukaryote , eukaryotic gene should be provided with necessary ingredients like prokaryotic promotor for expression, ribosomal binding sites for cloning vector . Large number of identical organisms that produce desired products are formed in prokaryotic cell. This can be identified by antigen antibody reaction and purified by standard chemical process.

21)List out the Source,role,and deficiency symptoms of Phosphorus, Potassium and magnesium.

Definition-½, Source-½,,Role-1,Deficiency symptoms-1 (2-½ each)

The essential elements which are required in large quantities by plants are called Major or macro nutrients .Ex: P ,K ,Mg, Ca , S,O , N .

A) Phosphorous. Source-phosphates such as HPO ,HPO phosphate ions. **Role-** 1)It is vital component of Nucleic acids, ATP ,NADP Phosphorylated compounds,phospholipids.2)Act as activator of enzymes 3) Healthy root growth, Translocation of carbohydrates . **Deficiency**

Symptoms- 1)premature leaf fall, become purple due to accumulation of Anthocyanin.2)Root & shoot become short, slender, retard flowering. 3) accumulation of carbohydrates, soluble nitrogen compounds.4)Reduction in rate of protein synthesis .5) Growth is retarded with necrotic patches on leaves and fruits.

B)Potassium. Source: soil minerals such as Biolite , Muscovite , Illite .**Role:**1)' K' is an activator of enzymes such as DNA Polymerase, starch polymerase.2) It regulates stomatal movement.3) Influence translocation and chlorophyll formation. 4) Maintain permeability and hydration.

Deficiency Symptoms;1)shoot become thin, stunted growth, Leading to death of plant .2) Inter veinal chlorosis, Necrosis at tips & margins of leaves. 3) Reduction in flowering. 4) Reduced storage of carbohydrates in under ground stem and root.

C) Magnesium .Source: Mg occurs as silicates, carbonates in soil and minerals such as magnesite, dolomite ,olvine. **Role ;**1) Mg is an important constituent of chlorophyll.2) play vital role in phosphorous metabolism.3) It is binding agent of ribosomal particles during protein synthesis .4) it acts as an activator for many enzymes like carboxylase, Hexokinase, Phosphorylase, dehydrogenase Peptidase(enzymes of carbohydrate metabolism & nucleic acid synthesis) .

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Deficiency Symptoms : 1)leaves develop Anthocyanin pigment & necrotic spots. 2) Petiole become slender and defoliation occurs.3) Reduction in size of the chloroplast .

XX

VI Semester B.Sc. Examination ,May/June 2013 Botany Paper – VII [Molecular Biology
,Genetic engineering, Biotechnology , plant physiology -1]

Scheme of valuation

A . Answer any Six of the following:

6x 2 = 12

9) **Mention any 4 characteristics of RNA.**

½ mark each.

RNA is a nucleic acid 90% is present in cytoplasm & rest in nucleus, single stranded polynucleotide composed of Ribose sugar, Phosphate, Nitrogen bases, Nitrogen bases are purines like Adenine, Guanine, and pyrimidines like Cytosine Uracil, Single strand is folded upon itself to form hair pin loops which show complementary base pairing, Base composition does not follow Chargoff's rule, It can't self replicate but synthesised from DNA .

10)

Define initiator codon. Name the amino

acid it codes . 1+ 1

The codon AUG that begins the protein synthesis is called Initiator codon. In Eukaryotes it codes for Methionine, In Prokaryotes it codes for N-Formyl methionine. Less frequently GUG is initiator codon & it codes for Valine.

11)

Mention 4 Applications of Genetic

Engineering in Horticulture. ½ each . Transgenic plants have been engineered by Scientists with special beneficial properties like A) Value addition to crops- Potato rich in starch, Soya bean that yields Cocoa oil, Tomato that ripen slowly etc, B) Herbicide ,Virus, Disease resistant plants-Tomato resistant to fruit worm,Papaya resistant to Ring spot virus etc,C) Ability to fix Nitrogen by non leguminous plants ,D) To alter flower quality, colours in Petunia Rose, Lotus, Chrysanthimum etc.

12)

What are Molecular Scissors ?Give an

example . 1+1

An Enzyme that recognise specific Palindromic sequence in DNA on both the strands and cut it with in the same recognition are called Molecular Scissors or Restriction endonuclease .EX : Eco RI , Hind II ,Hind III, Hpa II, Hae II,Taq I ,Hpa I Etc

13)

Differentiate between Plasmolysis and

Deplasmolysis. 1+1

Plasmolysis – Shrinkage of Protoplast due to Exosmosis when cell is

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present in hypertonic solution and cell become flaccid. Deplasmolysis – Regain of normal condition of protoplast in plasmolysed cell due to Endosmosis when cell is present in hypotonic solution and cell become Turgid.

14) What is Vein loading and Unloading ? 1+1

Vein loading –The movement of solutes from sites of photosynthesis into the Sieve elements of Phloem in vein. Vein unloading –The movement of solutes from Sieve elements of phloem into the receiver cells for consumption is called Vein unloading.

15) Differentiate between Transpiration and Guttation 1+1

Transpiration : Loss of water in vapour form through Stomata, Cuticle or Lenticel during day time. Transpired water is pure, gives cooling effect . Guttation: Loss of water in liquid form through Hydathode during night or early morning. Guttated water contain minerals, organic & inorganic substances , does not give cooling effect .

16) Ion Antagonism . Definition 1+ Example 1

Neutralising the toxic effect of particular element by maintaining ionic balance is called Ion Antagonism. Ex : For barely plant manganese concentration of 300-400 ppm is toxic, but it is neutralized when Silicon is present in the medium .

B . Answer any Six of the following :

6x4 =24

9) Griffth 's experiment :

1+1+1+1

1) Fredrich

griffth , a British medical officer(Bacteriologist) while working on pathogenicity of streptococcus pneumonia(it Cause pneumonia in mammals) observed in two strains of bacteria. They are :1)Virulent smooth strain(S) : It has polysaccharide capsule. 2) Avirulent Rough strains(R) : It lacks polysaccharide capsule.it produce rough colonies in culture .

2) Griffth showed that heat killed' S' strain when injected into experimental mice did not cause pneumonia .

3) When such heat

killed strain were injected to mice along with living' R' strain they died of pneumonia.

4) Autopsis showed presence of' S' strain instead of living R strain . According to Griffth some chemical present in' S' strain is responsible for change of' R' strain into' S 'strain he called it as "Transforming principle .

10) Explain Transcription

Fig- 1 ½ , Definition - ½ , Explanation -2

Synthesis of RNA

from DNA is called Transcription .

1) It starts at

promotor site on DNA, it is recognized by sigma factor of RNA polymerase and attaches to it . it is

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called Pribnow Box or TATAA Box

2)

DNA unwinds , Sense strand transcribes m-RNA . After initiation sigma factor is released from core enzyme

3)

chain elongation takes place 5'to 3' end by addition of nucleotides, A pair with U,T pair with A and G with C.

4)Termination site

is recognized by Rho factor , addition of nucleotides stops, NUS-A release new mRNA , DNA under goes coiling

11) Hazards of Genetic engineering .

8 hazards ½ each

1)Accidental escape of transformed microbes from laboratory into environment will pose great damage .2) this technique can be misused and unleash biological war fare on enemy countries .Ex: Air or water bodies might be sprayed with pathogenic microbes like plague, Anthrax etc.3) Genetically modified product may be toxic and threat to human health. Ex: L-tryptophan caused eosinophilia myalgia syndrome. 4) Genetically engineered weed variety of crop become super weed .5) Possibility of revival of fossil organisms and use of fossil DNA. 6) Genetic engineering patenting like terminator technology results in infertile seeds leading farmers for economic crisis. 7) Leads to global monopoly and affect Bio diversity.8) Genetic engineering research tinkers the basis of evolutionary process and disturb the fine tuned balance and relationship that is existing between organisms

12) Water potential and its components . Definition -1, Components-1+1+1.

Chemical potential of water is called water potential . In solvent system it represents difference between the chemical potential of water in that system and pure water .It is expressed by megapascals or bars and denoted by letter psi. water potential of pure water is zero .

In a plant water potential is sum total of 3 components .namely matric, solute and pressure potential.

- d) **Matric potential ()** : matric potential is defined as the amount by which water potential of the cell sap is reduced due to adsorption of water molecules by hydrophylic colloids of protoplasm . It is not significant as it does not allow free movement of water molecules.
- e) **Solute potential ()** : Solute potential is defined as the amount by which water potential of cell sap is reduced to solute particles present in it. It represent negative numbers.

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- f) **Pressure potential ()** : Pressure potential is defined as the amount by which water potential of the cell sap is reduced due to wall pressure (Pressure exerted by cell wall on cell contents) and Turgor pressure (equal pressure exerted by cell membrane on cell wall) . It represents positive sign.

13) Explain steps involved in penicillin production .

1+1+1+1

Penicillin is an Antibiotic extracted from penicillium notatum by Alexander Fleming. The yield of penicillin from p.notatum is very low and it could be easily destroyed by acid and heat. Hence now a days Penicillium chrysogenum is used for large scale production of penicillin by following steps:

- e) **Selection of strain** : High yielding varieties of p. chrysogenum are selected. They are genetically instable, hence maintained and stored carefully in frozen state in liquid nitrogen or fine suspension of spore is mixed with inert material like soil or sand and kept under desiccation.
- f) **Preparation of inoculum** : Pure inoculum of p.chrysogenum is developed to initiate fermentation using Moyer & Corgill culture medium, Nitrogen source is ammonium acetate, Ammonium sulphate, corn steep liquor supply potassium, dihydrogen phosphate, Magnesium sulphate.
- g) **Incubation** ; production tank is incubated using pressure to push 10% of inoculum into fermentation tank at 25- 26 c for 3-5 days. Periodic survey is done to check contamination if any and to determine quantity.
- h) **Harvest and recovery** : aseptically remove mycelium using Rotatory vacuum filter. Extract penicillium using counter current solvent extraction. Adjust PH of filtrate to acidic state (2- 2.5) using sulphuric acid. It is back extracted into an alkaline buffer with PH 7- 7.5. Thus crude penicillin is obtained. It is further treated with aqueous sodium hydroxide followed by charcoal to eliminate pyrogens and then filtered by Seitz filter to eliminate bacteria. For medicinal purpose dry powder is stored in vials.

14) Significance of osmosis.

8 uses ½ mark each

1. Osmosis helps in Absorption of water
- 2) Movement of water between cells in the plant body
- 3) Maintain shape, size posture, stature due to turgidity,
- 4) Seismonastic movement in mimosa pudica
- 5) Stomatal movement
- 6) Dehiscence of Sporangia & bursting of fruit
- 7) Develops

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resistance to drought , frost 8) Turgid cells of root tip easily penetrate in soil particles 9) Plasmolysis prevent the growth of moulds & bacteria in preserved food stuffs like pickles, jams.

15) Explain starch sugar interconversion hypothesis. Day -2 ,night-1 , obj-1

Starch Sugar inter conversion theory was proposed by Sayre in 1926, later modified by Steward in 1964. PH of the medium decides the inter conversion of starch and sugar results in stomatal movement. **During Day** time carbon dioxide liberated by respiration is used by mesophyll for photosynthesis, PH increases, insoluble starch is converted into Glucose -1-phosphate by phosphorylase, it is converted into Glucose -6-phosphate by phosphoglucomutase which is further converted into soluble Glucose and phosphate. concentration of cell sap increases, osmotic pressure increases, water diffuses into guard cell from subsidiary cells, Guard cell become turgid and stomata opens. **During night** carbon dioxide accumulates, form carbonic acid, PH decreases, Glucose is converted into Glucose -1-phosphate using ATP in presence of Hexokinase. It is converted into starch in presence of phosphorylase. concentration of cell sap decreases, water diffuses out of guard cell, turgor pressure decreases, guard cell become flaccid, stomata close. **Objections** 1) Monocots do not have starch 2) No evidence to show presence of sugar when starch disappears 3) Stomata close at mid day without change in starch conversion. 4) Stomatal movement do not require any energy.

16) Protoplasmic streaming hypothesis Expla-2, objections-1

This theory was proposed by Dutch Botanist Hugo de Vries, supported by O. F. Curtis. According to them, solute translocation is due to combination of Diffusion and protoplasmic streaming. Organic solutes translocate by protoplasmic streaming, when it reaches end of sieve tube migrates to the next sieve tube through sieve pores by diffusion. It explains bidirectional movement of metabolites. **Objections** : 1) According to Esau there is no cyclosis in sieve tube, even it exists in young decreases with maturity. 2) Phloem exudate is from vacuole and not from cytoplasm indicating solutes are transported through the vacuoles of sieve tubes.

C . Answer any Three of the following :

3x8= 24

17) Explain semi conservative method of DNA replication.

Definition-1, Fig, label- 3 , Explanation - 5

Replication or Duplication of DNA molecule means to make exact copies of its own structure. It takes place inside the chromosome during "S" phase of interphase. Semi

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conservative of DNA replication was proposed by Watson and Crick. In 1958, it was proved by M Meselson and F W Stahl . In this method ,**2 daughter molecules are formed ,each with one parental strand and a new strand** . It takes place as follows :

1)DNA Replication takes place at a specific point called ORI site or origin site.**2)** The DNA helix unwind with the help of DNA unwinding protein , the 2 strands separate from each other after breakage of hydrogen bonds between base pairs. Single strand binding protein extend along single strand and stabilize it. **3)**This results in Y shaped replication fork. The strain caused by unwinding is relieved by super helix relaxing protein. **4)**Initiation of DNA synthesis require RNA primer .It is a short polynucleotide chain synthesized by DNA template in presence of polymerase enzyme . The separated strands act as **Templates** . **5)**Synthesis of new DNA strand takes place by addition of nucleotides to 3-OH group of RNA primer in 5'- 3' direction in presence of DNA polymerase III .It take place continuously and it is referred as **Leading strand**.**6)**The other strand is synthesized in short fragments **on Lagging strand** .These are called **Okazaki fragments**. Named after discoverer Okazaki **7)** DNA Ligase joins okazaki fragments into long polynucleotide chain.**8)** DNA polymerase I degrades RNA primer by and simultaneously catalyses the synthesis of short DNA fragment to replace RNA primer . This segment is joined to main DNA strand by DNA ligase.**9)** The newly formed daughter strands are complementary to their template and undergo coiling with it.**10)**Thus 2 daughter DNA molecules are formed .Each daughter DNA has one parental strand and another new strand .Hence this method of DNA replication is called **Semiconservative method**.**11)** DNA duplication is a complex process carried by multi enzyme complex called **Replisome** .The segment of DNA under replication with its origin to termination is called "**Replicon**".

GENETIC ENGINEERING AND BIOTECHNOLOGY **14 Hrs**

A concise account of methods used in "Recombinant DNA technology".DNA (Gene) libraries, screening a genomic DNA library, Application of genetic engineering technology in Agriculture, Horticulture, and Floriculture. A brief account on hazards and safe guards of genetic engineering technology.

MICROBIAL BIOTECNOLOGY: uses of microbes in industry and Agriculture, fermentation, production of ethanol, Production of Enzymes-Amylase, Production of Antibiotics- Penicillin, Production of single cell protein-Spirulina.

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INTRODUCTION

The alteration of genotype of an organism using invitro techniques to produce products and services of human benefit is called "Genetic Engineering".

In 1973 Stanley Cohen (Stanford university. U.S.A) Herbert boyer (University of California) reported that desired gene can be isolated and joined into the plasmid of another organism using enzymes.

TOOLS USED IN GENETIC ENGINEERING

Biological tools used in genetic engineering are:1) Vector 2) Enzymes 3) Desired Gene 4) Host cell 5) Bio reactor.

I Vector:

Vectors are DNA molecules. The desirable DNA molecule is inserted into the host cell and made to replicate inside host cell. It is also called '**Cloning Vector' or 'Cloning vehicle'**'.

Vectors may be plasmids, Bacteriophage, Cosmids, Phagemids, Transposons. A vector must possess following characteristics:-

- Vector must replicate in host cell after its introduction.
- A unique cleavage site must be present in one of the marker genes.
- It must contain marker gene such as Resistance for Tetracycline, (TetR), Kanamycin(KanR), Ampicillin(AmpR).
- It should contain specific control systems like promoters,terminators, Ribosome binding site.

Plasmid: Plasmids are the extra chromosomal, self-replicating, double stranded, closed and circular, DNA molecules present in Bacterial cell. In 1973 Cohen et al reported Cloning DNA by using plasmid as vector.

A Plasmid can consider a suitable cloning vehicle if it shows following feature: -

- Plasmid can be easily isolated from the cells.
- It contain single restriction site for one or more restriction enzyme.
- Insertion of foreign gene should not alter replication properties of plasmids.
- It can be reintroduced into bacterial cell and shows normal activity in the parental cell.

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Based on the functions plasmids are classified into 5 types as follows:-

- 'F' Plasmid:- The plasmid with fertility or Sex factor 'F' is called 'F' plasmid .It is responsible for transfer of DNA during Conjugation.
- 'R' plasmid:- The plasmid with drug resistant and plasmid transfer gene is called 'R' plasmid.it makes cell resistance to Antibiotics.
- 'col' plasmid:- The plasmid with genes that directs the synthesis of proteins 'Colicin' are called 'Col'plamsid. These kill closely related strains that lack col plasmid.
- Virulence plasmid:- The plasmid with genes that cause virulence on the host bacterium are called Virulence plasmid. Ex : Ti plasmid found in Agrobacterium tumificans induce crown gall disease in dicot plants.
- Degradative plasmid:- The plasmid with genes that direct the catabolism of complex organic molecules are called 'Degradative or metabolic plasmid'.

Plasmids which occur naturally do not possess all the characteristics to use as cloning vector. Hence plasmids are constructed by inserting the genes for replication and antibiotic resistance.

PBR 322

The PBR 322 is the first artificial cloning vector developed in 1977 by Boliver and Rodrigueg from Escherichia coli.

- PBR 322 contains origin of replication.
- It contains genes for resistance to antibiotics like Ampicillin, Tetracyclin.
- It has unique recognition sites for 20 restriction endonucleases.
- When desired gene is inserted in tetr is inactivated .This is called Insertional activity.

In 1983 Messings and co workers developed PUC vectors at the University of California. They are PUC8, PUC 9, PUC 12, PUC 13, PUC 18, and PUC19.These consists of ori gene, Amp R, Lac z gene. The lac z gene consists of multiple cloning sites.

II ENZYMES

In genetic engineering Enzymes are used as Biological tools to cut and link DNA molecules. Some of them are as follows:-

1. **Exonuclease** : It act upon the genetic material , cleaves the 5' or 3' end of DNA.

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2. **Endonuclease** : It act upon the genetic material , cleaves one strand of the double stranded DNA at any points except ends.
3. **Restriction Endonuclease (REN)**: In 1970 Hamilton discovered REN in the bacterium Haemophilus influenza.

REN recognize specific DNA sequence on both the strands and cut within the same recognition site which are palindromic (Palindromic nucleotides are sequences that reads same forward and backward). REN are also called '**Molecular scissors**'.

REN cleave the DNA at recognition points. The cut ends posses short single stranded free ends called sticky ends. It can join with similar complementary ends of DNA from any source.

The REN are named based on following principles:

- REN is given three letter code written in italics.
- First letter is written in capital and it refers to genus name of bacteria from which it is obtained.
- Later two letters represent species name.
- Next letter indicate strain.
- Roman numerals represent different number of REN derived from same organism.
- EX: 1) **E Co R I** = **E**=Escherichia, **Co**=Coli, **R**= Strain, **I**= number.
2) **E Co R II** = **E**=Escherichia, **Co**=Coli, **R**= Strain, **II**= second Enzyme from the same bacterium
3) **Hind III** = **H**= Haemophilous, **in**= Influenza, **d**=strain, **III**= third enzyme from an organism.

4. **Ligase: (DNA Ligase)** : Ligase is a common joining enzyme obtained from Escherichia coli. These join any two cut ends of DNA, hence, they are commonly called 'Molecular Glue' or Molecular stichers'. In genetic engineering Ligase is used to join the desired gene to the DNA of the Vector.

III DESIRED GENE

The gene of interest like 'Nif 'gene, 'Bt' gene for growth of plant hormone , insulin secretion etc. that produce desired product is called " desired gene". It is identified, isolated, inserted into the plasmid. It forms the recombinant DNA.

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IV HOST CELL

The commonly used host cell for cloning desired gene is Bacteria Escherichia coli. It is prokaryotic, Gram--ve, non-spore producing, non capsulated bacillus form. They are found in soil, water, mouth, gut and help to protect intestinal track from infection. They form small amount of vitamin B 12 and K.E. coli can be cultured in nutrient medium at 37degree C. Yeast can also be used as host.

V BIO REACTOR

Bioreactor is an apparatus used in Biotechnological production, for growing organisms.

Bioreactor is made up of steel tank fitted with microprocessor and control unit. It controls PH, Dissolved oxygen, Gas flow rate, agitation speed, nutrients, inside temperature, density.

Bioreactors also contain sensors like PH, Temperature, Nutrient concentration,. An inlet for nutrients, steam, filtered air etc. It is covered with sight glass .The Vessel is covered with cold water both with inlet and out let to maintain water flow.

RECOMBINANT DNA TECHNOLOGY (r DNA)

The technology by which desired DNA fragment inserted into a plasmid is transferred into a host and activates it to produce desired product is called “Recombinant DNA technology”.

The process of rDNA technology involves following steps:-

- Isolation of desired gene from the donor.
- Insertion of desired gene into plasmid to form r plasmid.
- Transfer of r DNA (r plasmid) into a host.
- Culture of transformed cells to synthesize desired gene product.

1. Isolation of desired Gene

The gene that produce desired product is identified, isolated by refrigerated centrifuge technique. If desired gene is Eukaryotic, gene is obtained by “c DNA technique” (reverse transcription).

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Invitro synthesis of DNA from m-RNA by action of reverse transcriptase is called '**Reverse transcription**'. In 1970 Temin, Dulbecco, Baltimore discovered reverse transcriptase in retro virus. In 1975 they were awarded Nobel Prize.

The process of synthesis of cDNA from m-RNA involves following steps:-

- M-RNA is passed through an oligo-dI cellulose affinity column. It binds to poly-A-tail and provide free OH site for Reverse Transcription.
- Reverse transcriptase add complementary deoxy ribose nucleotide (dNTPs) one by one to 3'-OH site and form single stranded DNA.
- Terminal transferase, dCTP synthesize a short hair pin loop at 3' of cDNA.
- On hydrolysis using alkaline sucrose m-RNA separates from cDNA.
- cDNA acts as a Template and synthesise double stranded DNA in presence of DNA polymerase I.
- SI nuclease cleaves hair pin loop. Thus double stranded cDNA is formed.

2. Insertion of Desired gene into a plasmid

Plasmid is a circular, double stranded DNA found in bacterial cell which is used as vector for cloning desired gene. It is isolated from bacterial cell by treating with lysozyme(rupture cell wall) and centrifugation.(separate from other cell components).

Insertion of desired gene into plasmid involves following steps:-

- A Plasmid is cut with REN .It produces DNA fragment with sticky or blunt ends.
- The plasmid and desired gene are mixed together. Sticky end of plasmid and desired gene get linked by complementary base pairs.
- Enzyme Ligase seals the nick between plasmid and desired gene. Thus Recombinant DNA (rDNA) is obtained.

3. Transfer of rDNA into the host cell

There are several methods to transfer rDNA into host cell. The method depends on type of vector and host cells. Few methods are as follows:-Transformation, Electroporation, Shot gun method etc. In Transformaton strains of E.coli bacteria are pretreated with calcium chloride at low temperature and rDNAmixed up with it. rDNA migrate into E.coli.

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4. Culture of transformed cells

Host cells with recombinant DNA are identified and separated. This process is called Selection or Screening. Selected cells are grown with suitable nutrient media in Bioreactor. It produces large number of identical organisms or molecules. This is called 'Cloning'. The cloned gene rDNA produces desired product in an organism.

GENE LIBRARY

The collection of different DNA sequence from an organism, where each gene is cloned into a vector for purification, Storage & analysis is called "Gene Library".

Based on the source of DNA used, Gene library is of 2 types. They are as follows:-

1. **Genomic Library**
2. **cDNA library**

1. Genomic Library

Collection of all genes of an organism is called 'Genomic library'. Or obtained by 'Shotgun cloning technique'. It involves following steps:-

'Gene bank'. It is

A) Isolation and fragmentation of genome of a cell:

A cell of an organism contains all genes of an organism. The DNA of the cell is isolated and cut into fragments by addition of REN.

B) Isolation of Vector:

Plasmid vector present in bacteria is isolated, cut with endonuclease.

C) Insertion of Donor DNA into plasmid:

DNA fragments are inserted into plasmid, fused with it by ligase to produce rDNA.

D) Insertion of rDNA into Host cell:

rDNA are introduced into host cell E.coli by bacterial transformation.

E) Cloning the host cells:

Host cells are cultured in an agar plate. Each colony contains particular gene of donor organism.

G) Identification of cloned genes:

Cloned genes are identified, selected from colonies by immunochemical or colony hybridization technique.

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Thus identified and isolated genes from genomic library of particular organism.

cDNA Library or DNA Library

A collection of cDNA molecules for different characters made from m-RNA by the Reverse transcription is known as 'cDNA'(Complementary library) 'DNA library'. It represents DNA of only Eukaryotes.

In Eukaryotes heterogenous RNA is produced which contain coding sequence called 'Exons'.and non coding regions'Introns'.From this m-RNA , cDNA is synthesized by reverse transcriptase, Deoxyribo nucleotides. Etc.

Eukaryotic cells, number of m-RNA are involved in expression of many characters. Some number of cDNA molecules for different characters can be obtained. This constitutes DNA Library.

APPLICATION OF GENETIC ENGINEERING IN AGRICULTURE **HORTICULTURE, FLORICULTURE**

The process of production of desired plants for goods and services of mankind by incorporating desired genes through genetic engineering methods is called 'Tran geneses. The plants with inserted foreign genes are called 'Transgenic plants'.

Plant scientists have developed transgenic plants in Agriculture, Horticulture and floriculture with the objective as follows:

- I. To increase value addition to crop.**
- II. Disease, Herbicide, insect, Virus resistant plants.**
- III. Alter flower colors.**
- IV. Ability to fix Nitrogen by non-leguminous plants.**

I. Production of value added crops:

- **Vitamin-A rich Rice (Golden rice)** by transferring of Vitamin A producing genes.

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- Genetic engineered **French bean** with storage protein phaseolin.
- **Transgenic potato** with Starch and amino acid. By transferring AMAI protein cDNA of Amaranthus.
- Genetically engineered **Rape seed** rich in Serates and oleic acid (by transferring 9 stearoyl ACP desaturase .(It has stability during frying and no cholesterol).
- Genetically engineered **Soya bean** yield cocoa oil. It is made by transferring of genes for enzymes responsible for cocoa oil.
- **Transgenic tomato** (Flavo servu) is Brusie resistant and ripen slowly is achieved by transfer of antisense producing gene for polyalacturonase,
- **Transgenic pea** contain Sulphur rich amino acids
- **Transgenic cereals** contain high essential amino acids such as Lysine.
- **Trnasgenic plants** contain gene for antibody producton and yield edible vaccine .Ex: edible vaccine for hepatitis-B from Banana, Spinach or Tobacco for rabies.

II. Production of resistant plants:

- Herbicide resistant transgenic plant produced by introducing herbicide tolerant gene is ecofriendly, environmentally safe, detoxify herbicide and tolerate the effects.
- Insect resistant transgenic plants reduce use of chemical pesticides in Agriculture.ex: Bt-Cotton obtained from transfer of gene for endotoxin called Bt-cotton from bacteria Bacillus thurengensis is resistant to Boll worm. Tomato is resistant to tomato fruit worm.
- Virus resistant transgenic plants developed from by introducing gene that code for viral protein .Ex: Transgenic Tobacco is resistant to TMV Genetic engineered Potato resistant to Potato virus x, y. Genetic engineered Papaya resistant to papaya ring spot virus.

III. To alter Flower color:

Transgenic plants with attractive colors are produced by introducing genes involved in falconoid metabolism or antisense RNA producing genes .Ex: petunia.

IV. Transgenic plants as Bioreactors:

Transgenic plants are used as plant Bioreactors for large scale production of valuable products like vaccines, interferons, Biodegradable plastics.

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HAZARDS AND SAFE GUARDS OF GENETIC ENGINEERING

Research with recombinant DNA provides major new social benefits of greater magnitude .at the same time it has raised many fears that cannot be ignored. Hence perfect safeguards are required to carry on with application of Genetic engineering.

HAZARDS OF GENETIC ENGINEERING

1. Genetically modified organisms (GMS) used as Food can cause Allergic reactions.
2. GMO can be used as Bio weapons, initiating Bio war .EX; Pathogens of small pox, Anthrax, Botulinum toxin.
3. Genetically modified products may have toxins that are threat to human health .Ex: In 1989 genetically engineered product L-Tryptophan caused death and painful disorder eosinophilia malgia syndrome. (It was due to contamination of bacteria during recombinant DNA process).
4. Use of Recombinant Bovine growth hormone causes breast, prostate, Colon cancer in human beings.
5. Genetically engineered weed resistant variety of crop become super weed.Ex: Rape seed (canola) has spread its herbicide resistant to related weeds.
6. Possibility of revival of fossil organisms and use of fossil DNA .Ex: yeast of Jurassic age has been brought back to life. it is used to make beer.
7. Genetic pollution of organic and non genetic engineered crops may take place by transfer of genetically altered pollen through pollinating agents.
8. Genetic engineering patenting such as terminator technology results in infertile seeds. Thus former faces economic crisis due to expensive genetically engineered seeds.
9. It leads to Global monopoly and affect Biodiversity.
10. Pathogen DNA used as vaccine may enter other normal organism which would become new pathogen causing old disease.

SAFE GUAD OF GENETIC ENGINEERING

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The altered changes in the genetically engineered organisms may be fore seen problem concerned to human health, Socio-economic well being. Hence there is need for Bio-safety. Safety measures include following methods:

1. Accidental release of microbes from laboratory must be prevented.
2. Ban of dangerous experiments leading to Bio weapons.
3. Genetically microbes must be altered so that they cannot survive outside the laboratory.
4. Precautions should be taken during cloning of genes that code for cancer, toxin and antibiotics.
5. Mouth pipetting, Eating, drinking, storing food, applying cosmetics ,is prohibited.
6. Work places must be kept clean, must decontaminated daily.

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MICROBIAL BIOTECHNOLOGY

PRODUCTION OF ENZYMES –ETHANOL

Louis Pasteur demonstrated the fermentation of sugar by micro organisms. In 1815 Gay-Lussac formulated conversion of Glucose to ethanol. Recently recombinant DNA technology has helped micro –organisms used in industrial process. India is the third largest producer of fermentation ethanol.

Ethanol is used as Solvent, extract, Antifreeze, as substrates for synthesis of Dyes, Lubricants, Pharmaceutics, detergents, Pesticides, Explosives, Resins, Plasticizers, manufacture of synthetic fiber, as liquid fuel in the name of “Gasohol” and as Alcoholic beverage.

Micro organisms used : Bacteria such as Clostridium acetobutyllum, klebsiella pneumonia Fungi such as Aspergillus oryzae, Schharomyces cerevisiae (yeast).

Fermentation of ethanol is carried out in a large fermentor. The inoculums of micro organisms are maintained at optimum growth conditions like Temperature, pH, Oxygen, concentration of substrate, carbohydrate.

Preperation of inoculums

Fermentation process requires huge quantity of microbial culture. Medium has to be agitated and aerated for production of large amount of cell mass. PH range is 4.8 to 5 is optimum & temperature range between 20 to 30 degrees is optimum.

Preparation of inoculums for yeast culture involves following steps:

1. Take 25 ml of sterile culture medium in a test tube, add culture of yeast, and incubate at 28 to 30 degrees C for appropriate time.
2. Transfer the culture to conical flask containing 250 ml of sterile medium; incubate at 28 to 30 degrees C for appropriate time to get required quantity of culture.
3. Transfer the culture to large container to accommodate 5 liters of culture medium, incubate at 28 to 30 degrees for suitable time to obtain hug ecell mass.
4. Transfer the culture to a small tank that contain 50 to 200 liters of culture medium, incubate and then transfer to actual fermentor by pumping or gravity. The method of adding inoculums to the fermentor is called Pitching.

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Raw materials required for fermentation

Plant based substrates which contain starch or Sugar are used as raw materials to begin fermentation. They can be grouped as follows:

1. Roots, Tubers or Grains such as Potato Starch, Corn starch, Wheat flour, Cereals like oats, Barley etc.
2. Sugary materials such as Molasses, or Juice from Sugar cane, Sugar beet etc.
3. Wood or Waste products from processed wood.

Media preparation for fermentation

In India Molasses, Substrates containing sugars, Starch and cellulose are used as raw materials. Yeast do not contain Amylase, hence starch has to be hydrolysed (Saccharification) to form Glucose and Maltose. [In Saccharification

Starchy roots are ground, squeezed, sun dried. Starch is liquified, put under pressure, hydrolysed by adding enzymes. For fermentation 10 to 18 percent concentration of sugars is essential. It can be measured with Balling hydrometer.] In modern plants liquefaction and Saccharification is carried out by using steam injection and vacuum suction.

The following conditions are to be controlled to produce optimum quantity of Ethanol.

1. Nitrogen is very important .As nitrogen source Ammonium sulphate is added in the quantity of 0.15 g per 15 liters of molasses. Excess of nitrogen will inhibit fermentation.
2. Carbon compounds of 10 to 18 percent is satisfactory . high concentration affects growth of yeast and low conc. Reduces rate of fermentation.
3. pH: pH range of 4.8 to 5 is required. Higher ph increases contamination and lower pH reduces content of ethanol.
4. Temperature: Temperature of 72 to 80 F is preferable. Fermentation increases temp. To reduce temperatures cooling coils or cold waer spray are used.
5. Agitation: Agitation of the medium uniformly cools the medium.
6. Time: Fermentation begins few hours after addition of yeast culture. Production of Ethanol begins after 32 to 72 hours after initial process. When specified gravity of fermenting liquid called Wash becomes constant, it contains 6 to 8 percent of ethanol .5 liters of Molasses contains 2 liters of ethanol.

Recovery of Ethanol

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After fermentation Wash is allowed to settle down, and then subjected to fractional distillation in analyzer and rectifier columns to obtain ethyl alcohol.

Byproducts of ethanol fermentation

Apart from Ethanol 3 important byproducts are formed. They are:

Carbon dioxide: It is liquefied or converted into Dry ice, used commercially.

Distillary effluents: Alcohols produced as byproducts, after refining, used in perfumes and other industries. By esterification various types of acetates (n- propyl acetate, isopropyl acetate, amyl acetate) which are used in paint and liquor industry are formed. Yeast cell mass: It is a valuable fodder .

Alcoholic beverages:

Percentage of Alcohol differs in different alcoholic beverages. Ethanol is used in Alcoholic beverages.

Wine: Alcohol percentage is 10-22. European drink produced from juice of fresh grapes.

Brandy (Fortified wine): Alcoholic percentage is 20. Prepared from addition of extra ethanol to wine.

Beer: Alcoholic percentage is 4 to 8. Produced after fermentation of mixture of Barley, malt and starchy solution by yeast.

Rum: Alcohol percentage is 51. It is a distilled product of culture fluid. (Culture medium is prepared from Black strap molasses containing 12 to 14 % fermentable sugar, Ammonium Sulphate, Phosphates. After fermentation, culture fluid is distilled to remove alcohol which is used as Rum).

Whisky: Alcohol percentage is 51 to 59. It is prepared through fermentation of grain mash. Culture fluid contains Alcohol, esters.

PRODUCTION OF ENZYME-AMYLASE

Introduction

Enzymes are Biological catalysts that carry out metabolic conversions within the body of an organism at low temperature.

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In 1894 industrially first Enzyme Amylase was produced from fungal sources. In 1915 Otto Roehm produced first detergent enzyme for cleaning laundry clothes. By 1969 all detergents contained enzymes Proteases that digest protein impurities, Lipases, Amylases, Pectinases etc.

Amylases are enzymes that digest starch and produce sugars by the process of hydrolysis. Important enzymes in the breakdown of starch and production of sugars are alpha amylases, beta amylases, glucoamylases etc.

Alpha Amylases are group of endo enzymes that are characterized by the ability to hydrolyse 1-4 glycosidic linkage in polysaccharides like starch and glycogen. Beta amylases break down starch and amylases producing molecules of maltose.

Microorganisms Fungi such as *Aspergillus niger* , *Aspergillus oryzae* and bacteria such as *Bacillus amyloliquefaciens*, *Bacillus licheniformis* are employed in the production of Amyloses. Hence based on the microbes used amylases are called fungal amylase or Bacterial amylase.

Fungal Amylases

Fungal amylases can be produced by grains such as Wheat bran or Corn starch when culture is employed in semisolid culture. In this process Wheat bran is spread in the form of thin layers in trays or in rotary drum fermentors and water is added to soak grains. Fungal spore of *Aspergillus niger* is inoculated, after the growth of fungal mycelium entire medium is dried at 50 degrees C. and ground to obtain the crude amylase .aqueous extract of amylase is precipitated by addition of alcohol and dried at 55 degrees C.

Bacterial amylases

Commercial scale production of Amylase is done by *Bacillus Licheniformis* , *B. amyloquefaciens*. It has better degree of temperature tolerance than antifungal amylases and is active even at 55 degree C.

Bacteria which have high liqifying and dextrifying activity are selected, pH is maintained at around 7, fermentation should continue for six days at temperature ranging between 25 to 30 degrees C. bacterium produces high surface growth, fresh air is circulated over surface to improve aeration. The culture is harvested by filtration or by centrifugation .filtrate or supernatant contains enzymes which further be concentrated by evaporation.

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Bacterial Amylases can also be produced by using highly aerated submerged cultures with the help of starch rich medium.

Uses of Amylase

Amylases are used as sizing agents and in the preparation of starch sizing paste for using in paper coatings and for liquefaction of heavy pastes in the manufacture of corn and chocolates, syrups. Laundry amylases are used for removal of food stains along with proteases.

PRODUCTION OF ANTIBIOTICS -Penicillin

Penicillin is an Antibiotic extracted from penicillium notatum by Alexander Fleming in 1929. Benzyl penicillin and penicillin v are natural antibiotics effective against Gram +ve bacteria. as they inhibit bacterial cell wall synthesis. But these are ineffective against microbes that produce B- lactanase, since they hydrolyse penicillin. the yield of penicillin from p.notatum is very low and it could be easily destroyed by acid and heat.

To overcome such problems semisynthetic penicillins are developed and used against gram—ve bacteria .Hence now a day's Penicillium chrysogenum is used for large scale production of penicillin by following steps:

- a) **Selection of strain:** High yielding varieties of p. chrysogenum are selected. They are genetically instable, hence maintained and stored carefully in frozen state in liquid nitrogen or fine suspension of spore, mixed with inert material like soil or sand and kept under desiccation.
- b) **Preparation of inoculum :** Pure inoculum of p.chrysogenum is developed to initiate fermentation using Moyer & Corgill culture medium ,Nitrogen source is ammonium acetate, Ammonium sulphate , corn steep liquor supply potassium, dihydrogen phosphate, Magnesium sulphate.
- c) **Incubation ;** production tank is incubated using pressure to push 10% of inoculum into fermentation tank at 25- 26 c for 3-5 days. Periodic survey is done to check contamination if any and to determine quantity.

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d) **Harvest and recovery:** aseptically remove mycelium using Rotatory vacuum filter. extract penicillium using counter current solvent extraction .Adjust PH of filtrate to acidic state (2.5) using sulphuric acid. It is back extracted into an alkaline buffer with PH 7- 7.5 .Thus crude penicillin is obtained .It is further treated with aqueous sodium hydroxide followed by charcoal to eliminate pyrogens and then filtered by Seitz filter to eliminate bacteria. For medicinal purpose dry powder is stored in vials.

Production of Single cell Protein (SCP)

The dried cells of Micro-organisms like layer Algaem bacteria, Fungi used as food are collectively known as Microbial proteins. In 1967 in the first international conference on “Microbial protein” was held at Massachusetts. The Microbial protein was replaced by **‘Single cell protein’ (SCP).**

Production of Single cell protein (SCP).

Spirulina is a unicellular, microscopic, and filamentous; Cyanobacteria It is longed and coiled.

Cultivation of Spirulina

Mass production of Spirulina is carried out by 2 types .They are:
Semi natural lake system. 2. Artificially built cultivation system.

1.

Semi natural lake system

Sosa Texcoco lake in Mexico and lake Chad in Africa provide a good environment for the natural growth of Spirulina . SCP obtained from these lakes is of low quality due to contamination and pollution , but provides a good food for fishes and animals.

1. Artificially built cultivation system

Based on the water quality and the nutrient status artificially built cultivation system can be grouped into 2 types. They are:

a) **Clean water system**

b) **Waste water system.**

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a) Clean water system

In clean water system artificial cultivation farm is constructed. These have shallow race way ponds circulated with paddle and high quality nutrients. To promote growth of algae NaNO_3 and NaHCO_3 is added. PH is initially maintained at 8.5. Spirulina is self pH adjusting alga which elevates pH between 10 to 10.5 at which there will be no contamination. India has 2 centers. Namely

1. Central food technological research institute (CFTRI) at Mysore.
2. Murugappa chettiar research center at Chennai. It produces about 75 tonnes of Spirulina annually.

b) Waste water system

In waste water system Human, Animal waste and sewage are used for growth of Spirulina. This can be applicable to highly populated countries where high quantities of wastes are generated and pose environmental problems.

In this system waste water is first added to digester which helps in setting of solid particles. Liquid effluents, NaNO_3 , NaHCO_3 are added as source of nutrition to artificially constructed ponds. Then Spirulina is added to this water. After sufficient growth, it is harvested from the pond, added to aquaculture to feed fish, or dried in small solar drier for human food.

Growth requirements for spirulina:

1. Algal tank: Circular or Rectangular cement tank with depth of 25 cm are constructed.
2. Light: Low light intensity is required to avoid photolysis.
3. Temperature: For optimum growth temperature should be 35 to 40 C
4. pH : pH ranging from 8.5 to 10.5
5. Agitation: Agitation is necessary for good quality, better yield. The culture is agitated by brush, paddle power, rotators. Wind power, pipe pumps.
6. Harvesting: Spirulina form thick mat over water surface. It can be harvested by fine mesh screen, Nylon or cotton cloth.
7. Drying: Sun drying gives good results.
8. Yeild: 8- 12 gms/ day is obtained in India. It is equal to 20 tonnes / annum.

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9. Avoid contamination: Dried powder is packed in Aluminium bags or sealed in bottles and sent to market.

Uses of Spirulina Single cell protein

1. As protein supplemented food:

Spirulina is rich in protein, vitamins, Amino acids, minerals, crude fibers etc. used as supplementary food for undernourished children. (1 gm of Spirulina tablets contain as much as one kg of vegetables).

2. As Health food:

Spirulina is a popular health food for instant energy, to control obesity, as it has very less calories.

3. In therapeutic and natural medicine:

Spirulina has many medicinal properties. It is recommended for reducing body weight, cholesterol, pre menstrual stress, for better health. In diabetic patients it reduces sugar level in blood due to presence of gamma –linolenic acid. Prevents accumulation of cholesterol in human body. B- Carotene helps in monitoring healthy eye and skin. *T increases lactation in nourishing mother.*

4. In cosmetics:

Spirulina is rich in vitamin A and B, known for healthy growth of hair. Herbal cosmetics are known to produce Spirulina based beauty products. Phycocyanin pigments present in it are used in biolipsticks and face creams.

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Practicals-VIII

1. Quantitative estimation of protein by Lowry's method
2. DNA isolation from onion/banana/ cauliflower
3. Quantitative estimation of DNA by DPA method
- 4 Quantitative estimation of RNA by Orcinol method
5. Study of PBR322, Northern, Southern and Western Blotting, DNA Fingerprinting and PCR by photographs
6. Gene data retrieval from NCBI
7. Estimation of chloride and dissolved oxygen in water sample.
8. *Spirulina* Cultivation
9. Visit to research institutes.
10. **A project work / dissertation work (related to Botany topic) / Tour report has to be Submitted for evaluation at the time of practical examination (Duly certified by the Supervising teacher and Head of the Department)**

Suggested Readings

1. De Robertis: Cell and Molecular Biology
2. Essentials of Molecular Biology: Freifelder, D. & Malacinski, G.M. 1998 (or latest edition)
3. Lewin, B. Genes VI, 1997, Oxford Univ. Press, Oxford, New York, Tokyo.
4. Cell and Molecular biology, Harvay Lodish, David Baltimore, Arnold Beek,
5. Biotechnology : P.D. Sharma
6. Biotechnology : R.C. Dubey. s. Chand & co
7. Molecular Biology: Verma and Agarwal. S Chand & co
8. Concepts in molecular biology: Rastogi V.B.
9. Elements of Biotechnology: Gupta .PK
10. Biotechnology: Satyanarayana.
11. Experiments in microbiology: Aneja. Vishwa prakashan , New delhi.
12. Bioinformatics: principles and applications; Ghosh Z. and Bibekanand M Oxford University Press
13. Campbell A. M., Heyer L. J. (2006) Discovering Genomics, Proteomics and Bioinformatics.

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Practical Examination - Paper – VIII

Time : 3 Hrs Max marks :50

*1. Estimate the protein content of the sample 'A' by Lowry's method

Or 15

Estimate the RNA content of the sample 'A' by Orcinol method.

Or

Estimate the DNA content of the sample 'A' by DPA method

2. Estimate the chloride / dissolved oxygen content in the given sample 'B' 10

3. Comment on 'C' and 'D' 2x5= 10

4. Project report /Dissertation work/Tour Report 5

5. Viva voce 5

6. Class record 5

Scheme of valuation

1. Requirements – 1 mark, principle – 1marks, procedure -4, conduction – 5 marks,
Calculation & result – 4 marks.

(* students should select one of the experiments 'A' by means of lottery chit)

(. Requirements – 1 mark, principle – 1marks, procedure -4, conduction – 3 marks,
Calculation & result – 1 marks

2. Identification -1 mark, diagram – 1 mark , comment – 3 marks.

Experiments: 1 PBR322,

2 Northern, Southern and Western Blotting,

3 DNA Fingerprinting

4 PCR

(By photographs)

4. Project report / dissertation work / tour report - 5 marks

5. Viva based on the experiments given in the examination. -5 marks

6. Class Records. 5 marks