A draft note on

INTRODUCTORY CROP PHYSIOLOGY

(For B. Sc. Ag 2\textsuperscript{nd} semester)

Course code: PPH 121. \textbf{Credit hours: 3(2+1)}

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1. Introduction

Definition, scope and practical application of crop physiology

Literal Definition:
“Discourse on the nature of plants”
- A vague & very broad definition -
My Definition:
“Science of how plants develop, grow, and respond to their environment at the cellular & biochemical level”

What is Plant Physiology?

A. Definitions (numerous) - Plant physiology is the study of:
   - the functions and processes occurring in plants
   - the vital processes occurring in plants
   - how plants work

B. In essence, plant physiology is a study of the plant way of life, which include various aspects of the plant lifestyle and survival including: metabolism, water relations, mineral nutrition, development, movement, irritability (response to the environment), organization, growth, and transport processes.

C. Plant physiology is a lab science.
D. Plant physiology is an experimental science.
E. Plant physiology relies heavily on chemistry and physics.

The term physiology is derived from latin word “ physis” meaning nature and “logos’ meaning science. Plant physiology is a subdiscipline of botany concerned with the functioning, or physiology, of plants. Closely related fields include plant morphology (structure of plants), plant ecology (interactions with the environment), phytochemistry (biochemistry of plants), cell biology, and molecular biology.

Fundamental processes such as photosynthesis, respiration, plant nutrition, plant hormone functions, tropisms, nastic movements, photoperiodism, photomorphogenesis, circadian rhythms, environmental stress physiology, seed germination, dormancy and stomata function and transpiration, both part of plant water relations, are studied by plant physiologists.

Julius von Sachs (October 2, 1832 - May 29, 1897), German botanist, was born in Breslau, Silesia. is considered as the father of Plant Physiology

Crop physiology can be discussed under the following aspects:

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• Physical aspects: biophysical phenomenon, soil water relation, absorption and translocation of water, transpiration, ascent of sap, guttation e.t.c.
• Metabolic aspects: enzymatic activities, photosynthesis, respiration, carbohydrate, protein, lipids and nitrate metabolism.
• Growth and development aspects: seed dormancy, vernalization, photoperiodism, seed germination, growth and development, growth hormones, flowering, senescence.

It has a vast scope due to food crisis, malnutrition, ecological imbalance, reduction of cultivated land and decrease in soil fertility.

Why Study Plant physiology?

1. Food - plants are the route by which solar energy enters ecosystems
2. Economically important products - plants produce countless products from fibers to medicines to wood. For example, you can check out the web notes for my Plants and Human Affairs course or The Society for Economic Botany
3. Applications to other disciplines (i.e., agriculture, forestry, horticulture)
4. Theoretical importance (like a mountain - it’s there!)
5. Jobs! - see career pamphlets in file box. Also, check out the web sites for the American Society of Plant Biologists, Botanical Society of America, American Phytopathology Society and others.
6. It's fun & exciting (but, I guess not everyone necessarily agrees!)
7. Botany without Borders is a good online film that highlights the importance of plants. It was created by Dr. K Niklas (Cornell)

Scope

Figure: Five key areas of study within plant physiology

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The field of plant physiology includes the study of all the internal activities of plants—those chemical and physical processes associated with life as they occur in plants. Major subdisciplines of plant physiology include phytochemistry (the study of the biochemistry of plants) and phytopathology (the study of disease in plants). The scope of plant physiology as a discipline may be divided into several major areas of research.

Plant physiology includes the study of biological and chemical processes of individual plant cells. That is it help to disclose complex life processes.

Plant physiology deals with interactions between cells, tissues, and organs within a plant. Plants have developed a number of features and the functioning of the various modes of transport is studied by plant physiologists.

Plant physiologists study the ways that plants control or regulate internal functions. Like animals, plants produce chemicals called hormones which are produced in one part of the plant to signal cells in another part of the plant to respond. Many flowering plants bloom at the appropriate time because of light-sensitive compounds that respond to the length of the night, a phenomenon known as photoperiodism. The ripening of fruit and loss of leaves in the winter are controlled in part by the production of the gas ethylene by the plant.

Plant physiology includes the study of how plants respond to conditions and variation in the environment, a field known as environmental physiology. Stress from water loss, changes in air chemistry, or crowding by other plants can lead to changes in the way a plant functions. These changes may be affected by genetic, chemical, and physical factors.

Several new biological sciences have been evolved such as biochemistry, biotechnology, molecular biology and genetic engineering for which deep knowledge of plant physiology is required.

Research and development of new sophisticated technologies that may be required in crop improvement process.

Employment opportunities.

**Practical application of crop physiology**

- The major goal of crop physiology is to study the mechanism and sequence of events that occur in plant during their various life processes, responses and function with a view point of improving plant growth in agriculture, horticulture and forestry.
- The most important application of crop physiology is to increase agricultural productivity.
- In horticulture and agriculture along with food science, plant physiology is an important topic relating to fruits, vegetables, and other consumable parts of plants. Topics studied include: climatic requirements, fruit drop, nutrition, ripening, fruit set. The production of food crops also hinges on the study of plant physiology covering such topics as Optimal planting and harvesting times and post harvest storage of plant products for human consumption and the production of secondary products like drugs and cosmetics.
- Understanding the biochemical processes is key to improving crop yields or modifying the physiology of a plant to make it more tolerant of diseases, pests, drought, or other adverse conditions.
• Also, genetic engineers can introduce genes for the production of important biomolecules such as the enzyme, rennet, used to make cheese. Furthermore, engineers may be able to improve photovoltaic devices to improve the production of electricity using solar energy by mimicking the structure of chloroplasts found in leaves.
• The control of soil fertility, pollution and mineral requirement is another field where the knowledge can be applied. Such as improvement in biological nitrogen fixation, selection of proper Rhizobium strains.
• The proper knowledge of soil-water-plant relationship will enhance the bumper yield of crops.
• Effective and efficient use of plant growth regulators for enhancing the growth and productivity is another application of physiological study.
• Crop physiologists have developed techniques of soil management, irrigation practices, seed treatment, weed and pest control, hormonal control of flowering, fruit set and fruit drops, seedless fruit production, increasing succulence and sugar content, green house cropping, soilless cropping, cold storage, gas storage, artificial ripening e.t.c
• Thus the role of crop physiologist is to integrate information to synthesize new level of knowledge so as to develop problem solving system interacting with all specialists of agriculture.
• Thus contribution of science of crop physiology in raising the standard of people across the globe is significant.

2. Cell physiology

2.1. Definition, type and ultra structure of a typical cell
2.2. Structure and function of various cell organelles

The cell is the basic structural and functional unit of all known living organisms. It is the smallest unit of life that is classified as a living thing, and is often called the building block of life. Organisms can be classified as unicellular (consisting of a single cell; including most bacteria) or multicellular (including plants and animals). Humans contain about 10 trillion ($10^{13}$) cells. Most plant and animal cells are between 1 and 100 µm and therefore are visible only under the microscope.

The cell was discovered by Robert Hooke in 1665. The cell theory, first developed in 1839 by Matthias Jakob Schleiden and Theodor Schwann, states that all organisms are composed of one or more cells, that all cells come from preexisting cells, that vital functions of an organism occur within cells, and that all cells contain the hereditary information necessary for regulating cell functions and for transmitting information to the next generation of cells.

The word cell comes from the Latin cella, meaning "small room". The descriptive term for the smallest living biological structure was coined by Robert Hooke in a book he published in 1665 when he compared the cork cells he saw through his microscope to the small rooms monks lived in.
Anatomy

On the basis of the absence of well-defined nucleus, living organisms were earlier classified into two groups by molecular biologist viz.

- **Prokaryotes:** Cells not having well defined nucleus as in bacteria and blue green algae.
- **Eukaryotes:** Included remaining type of organisms having well-organized nucleus.
### Differences Between Prokaryotic and Eukaryotic Cells

<table>
<thead>
<tr>
<th></th>
<th>Prokaryotic Cells</th>
<th>Eukaryotic Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>small cells (&lt; 5 mm)</td>
<td>larger cells (&gt; 10 mm)</td>
<td></td>
</tr>
<tr>
<td>always unicellular</td>
<td>often multicellular</td>
<td></td>
</tr>
<tr>
<td>no nucleus or any membrane-bound organelles, such as mitochondria</td>
<td>always have nucleus and other membrane-bound organelles</td>
<td></td>
</tr>
<tr>
<td>DNA is circular, without proteins</td>
<td>DNA is linear and associated with proteins to form chromatin</td>
<td></td>
</tr>
<tr>
<td>ribosomes are small (70S)</td>
<td>ribosomes are large (80S)</td>
<td></td>
</tr>
<tr>
<td>no cytoskeleton</td>
<td>always has a cytoskeleton</td>
<td></td>
</tr>
<tr>
<td>motility by rigid rotating flagellum made of flagellin</td>
<td>motility by flexible waving undulipodium, made of tubulin</td>
<td></td>
</tr>
<tr>
<td>cell division is by binary fission</td>
<td>cell division is by mitosis or meiosis</td>
<td></td>
</tr>
<tr>
<td>reproduction is always asexual</td>
<td>reproduction is asexual or sexual</td>
<td></td>
</tr>
<tr>
<td>huge variety of metabolic pathways</td>
<td>common metabolic pathways</td>
<td></td>
</tr>
</tbody>
</table>

#### Comparison chart

<table>
<thead>
<tr>
<th></th>
<th>Eukaryotic Cell</th>
<th>Prokaryotic Cell</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nucleolus:</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>Number of chromosomes:</td>
<td>More than one</td>
<td>One--but not true chromosome: Plasmids</td>
</tr>
<tr>
<td>Cell Type:</td>
<td>Multicellular</td>
<td>Unicellular</td>
</tr>
<tr>
<td>True Membrane bound Nucleus:</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>Example:</td>
<td>Animals and Plants</td>
<td>Bacteria and Archaea</td>
</tr>
<tr>
<td>Telomeres:</td>
<td>Present (Linear DNA)</td>
<td>Circular DNA doesn't need telomeres</td>
</tr>
<tr>
<td>Genetic Recombination:</td>
<td>Mitosis and fusion of gametes</td>
<td>Partial, undirectional transfers DNA</td>
</tr>
<tr>
<td>Lysosomes and peroxisomes:</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>Microtubules:</td>
<td>Present</td>
<td>Absent or rare</td>
</tr>
<tr>
<td>Endoplasmic reticulum:</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>Mitochondria:</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>Characteristics</td>
<td>Plant Cell</td>
<td>Animal Cell</td>
</tr>
<tr>
<td>-----------------</td>
<td>------------</td>
<td>-------------</td>
</tr>
<tr>
<td>Cell Size</td>
<td>Large</td>
<td>Smaller than plant cells</td>
</tr>
<tr>
<td>Cell Shape</td>
<td>Rectangular</td>
<td>Circular</td>
</tr>
<tr>
<td>Vacuoles</td>
<td>A single centrally located vacuole. It takes up almost 90% of the cell volume. The vacuole stores water and maintains turgidity of the cell.</td>
<td>If any, there are a number of small vacuoles spread throughout the cytoplasm that store water, ions and waste materials.</td>
</tr>
<tr>
<td>Cell Wall</td>
<td>A rigid cell wall (made of cellulose) is present around a plant cell that helps it maintain its shape.</td>
<td>Cell wall is absent. This allows animal cells to adopt different shapes.</td>
</tr>
<tr>
<td>Chloroplasts</td>
<td>Present. Chlorophyll is the pigment that traps sun's energy which is utilized by plants to make food through the process of photosynthesis. This pigment is present in the chloroplasts.</td>
<td>Absent. As animals lack this pigment, they cannot make their own food.</td>
</tr>
<tr>
<td>Cell Division</td>
<td>Cell division takes place by the formation of cell plate in the center</td>
<td>Animal cells divide with the formation of a cleavage furrow. This is formed as the...</td>
</tr>
</tbody>
</table>
of the dividing cell. This becomes the cell wall between the two daughter cells.

<table>
<thead>
<tr>
<th>Centrioles</th>
<th>Present only in lower forms. Plants instead have microtubule organizing centers (MTOC) that produce the microtubules.</th>
<th>Present. Centrioles help in division of animal cells by creating microtubule spindles that pull the chromosomes to opposite ends for cell division to occur.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Centrosome</td>
<td>Absent. Instead two small clear areas called polar caps are present.</td>
<td>Present.</td>
</tr>
<tr>
<td>Lysosomes</td>
<td>Absent</td>
<td>Present. Lysosomes are vesicles that contain enzymes that destroy dead cell organelles and other cells debris.</td>
</tr>
<tr>
<td>Golgi Bodies</td>
<td>In place of golgi bodies, its subunits known as dictyosomes are present.</td>
<td>Complex golgi bodies are present close to the nucleus.</td>
</tr>
</tbody>
</table>

**A. Plastids in plants**

In plants, plastids may differentiate into several forms, depending upon which function they play in the cell. Undifferentiated plastids (*proplastids*) may develop into any of the following variants:
- Chloroplasts: green plastids; for photosynthesis.
- Chromoplasts: coloured plastids; for pigment synthesis and storage.
- Gerontoplasts: control the dismantling of the photosynthetic apparatus during senescence.
- Leucoplasts: colourless plastids; for monoterpene synthesis; leucoplasts sometimes differentiate into more specialized plastids:
  - Amyloplasts: for starch storage and detecting gravity.
  - Elaioplasts: for storing fat.
  - Proteinoplast/aleuronoplasts: for storing and modifying protein.

**Chloroplasts** (/ˈklorəplɑːst/) are organelles found in plant cells and other eukaryotic organisms that conduct photosynthesis. Chloroplasts capture light energy, store it in the energy storage molecules ATP and NADPH and use it in the process called photosynthesis to make organic molecules and free oxygen from carbon dioxide and water.

Chemical composition: (on the basis of dry weight)

<table>
<thead>
<tr>
<th>S. No</th>
<th>Chemical</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Proteins</td>
<td>35 – 55%</td>
</tr>
<tr>
<td>2.</td>
<td>Lipids</td>
<td>20 – 30%</td>
</tr>
<tr>
<td>3.</td>
<td>Carbohydrates</td>
<td>Variable</td>
</tr>
<tr>
<td>4.</td>
<td>Chlorophyll</td>
<td>9%</td>
</tr>
<tr>
<td>5.</td>
<td>Carotenoids</td>
<td>4.5%</td>
</tr>
<tr>
<td>6.</td>
<td>RNA</td>
<td>3 – 4%</td>
</tr>
<tr>
<td>7.</td>
<td>DNA</td>
<td>0.5%</td>
</tr>
<tr>
<td>8.</td>
<td>Minerals</td>
<td>0.2%</td>
</tr>
</tbody>
</table>

**Structure**

- Chloroplasts are observable as flat discs usually 2 to 10 micrometers in diameter and 1 micrometer thick. In land plants, they are, in general, 5 μm in diameter and 2.3 μm thick. The chloroplast is contained by an envelope that consists of an inner and an outer phospholipid membrane. Between these two layers is the intermembrane space. A typical parenchyma cell contains about 10 to 100 chloroplasts.
- The material within the chloroplast is called the stroma, corresponding to the cytosol of the original bacterium, and contains one or more molecules of small circular DNA. It also contains ribosomes; however most of its proteins are encoded by genes contained in the host cell nucleus, with the protein products transported to the chloroplast.
Photosynthetic Pigments:

Pigments carrying out photosynthesis are called as photosynthetic pigments. Chloroplast also contains photosynthetic pigments. They are chlorophylls, carotenoids and phycobilins. Chlorophyll a absorbs well at a wavelength of about 400-450 nm and at 650-700 nm; chlorophyll b at 450-500 nm and at 600-650 nm. Xanthophyll absorbs well at 400-530 nm. However, none of the pigments absorbs well in the green-yellow region, which is responsible for the abundant green we see in nature.

2. Endoplasmic reticulum

The endoplasmic reticulum (ER) is an organelle of cells in eukaryotic organisms that forms an interconnected network of tubules, vesicles, and cisternae. Rough endoplasmic reticula are involved in the synthesis of proteins and is also a membrane factory for the cell, while smooth endoplasmic reticula are involved in the synthesis of lipids, including oils, phospholipids and steroids, metabolism of carbohydrates, regulation of calcium concentration and detoxification of drugs and poisons. The lacey membranes of the endoplasmic reticulum were first seen by Keith R. Porter, Albert Claude, and Ernest F. Fullam in the year 1945.

The surface of the rough endoplasmic reticulum (RER) is studded with protein-manufacturing ribosomes giving it a "rough" appearance.
The smooth endoplasmic reticulum (SER) has functions in several metabolic processes, including synthesis of lipids and steroids, metabolism of carbohydrates, regulation of calcium concentration, drug detoxification, attachment of receptors on cell membrane proteins, and steroid metabolism.

**Functions**

The endoplasmic reticulum serves many general functions, including the facilitation of protein folding and the transport of synthesized proteins in sacs called cisternae.

- Proteins that are transported by the endoplasmic reticulum and from there throughout the cell are marked with an address tag called a signal sequence.
- The endoplasmic reticulum is also part of a protein sorting pathway. It is, in essence, the transportation system of the eukaryotic cell.

**Other functions**

- **Insertion of proteins into the endoplasmic reticulum membrane:** Integral membrane proteins are inserted into the endoplasmic reticulum membrane as they are being synthesized (co-translational translocation). Insertion into the endoplasmic reticulum membrane requires the correct topogenic signal sequences in the protein.
- **Glycosylation:** Glycosylation involves the attachment of oligosaccharides.
- **Disulfide bond formation and rearrangement:** Disulfide bonds stabilize the tertiary and quaternary structure of many proteins.
- **Drug metabolism:** The smooth ER is the site at which some drugs are modified by microsomal enzymes, which include the cytochrome P450 enzymes.

3. **Mitochondria** are present in both plant and animal cells. They are rod-shaped structures that are enclosed within two membranes - the outer membrane and the inner membrane. The membranes are made up of phospholipids and proteins. The space in between the two membranes is called the inter-membrane space which has the same composition as the cytoplasm of the cell. However, the protein content in this space differs from that in the cytoplasm.

**Composition:**
Protein: 65-70%, lipids: 25-30%, RNA: 0.5-2%, small amount of DNA, several types of enzymes.

The structure of the various components of mitochondria are as follows:

- **Outer Membrane**
  The outer membrane is smooth unlike the inner membrane and has almost the same amount of phospholipids as proteins. The

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outer membrane is completely permeable to nutrient molecules, ions, ATP and ADP molecules.

- **Inner Membrane**
  The inner membrane is more complex in structure than the outer membrane as it contains the complexes of the electron transport chain and the ATP synthetase complex. It is permeable only to oxygen, carbon dioxide and water. It is made up of a large number of proteins that play an important role in producing ATP, and also helps in regulating transfer of metabolites across the membrane. The inner membrane has infoldings called the **cristae** that increase the surface area for the complexes and proteins that aid in the production of ATP, the energy rich molecules.

- **Matrix**
  The matrix is a complex mixture of enzymes that are important for the synthesis of ATP molecules, special mitochondrial ribosomes, tRNAs and the mitochondrial DNA. Besides these, it has oxygen, carbon dioxide and other recyclable intermediates.

Functions of mitochondria vary according to the cell type in which they are present.

- The most important function of the mitochondria is to produce energy. This entire process is known as oxidative phosphorylation.
- It is important to maintain proper concentration of calcium ions within the various compartments of the cell.
- They also help in the building of certain parts of the blood, and hormones like testosterone and estrogen.
- Mitochondria in the liver cells have enzymes that detoxify ammonia.
- Kreb cycle takes place in matrix of mitochondria.
- It is involved in photo respiration.
- **Synthesis of steroid hormones:** The early step in the conversion of cholesterol to steroid hormones in the adrenal cortex, are catalyzed by mitochondrial enzymes.
- **Urea cycle:** Urea cycle plays crucial role in ammonia detoxification. In this cycle, urea is synthesized. The first step of this cycle that is the conversion of ornithine to citrulline occurs in the mitochondria.

**4. Ribosome**

The **ribosome** (from ribonucleic acid and the Greek *soma*, meaning "body") is a large complex of RNA and protein which catalyzes protein translation, the formation of proteins from individual amino acids using messenger RNA as a template. This process is known as translation. Ribosomes are found in all living cells.
Structure

The ribosomal subunits of prokaryotes and eukaryotes are quite similar. The unit of measurement is the Svedberg unit, a measure of the rate of sedimentation in centrifugation rather than size and accounts for why fragment names do not add up (70S is made of 50S and 30S).

Prokaryotes have 70S ribosomes, each consisting of a small (30S) and a large (50S) subunit. Eukaryotes have 80S ribosomes, each consisting of a small (40S) and large (60S) subunit.

Function

- Ribosomes are the workhorses of protein biosynthesis, the process of translating mRNA into protein. The mRNA comprises a series of codons that dictate to the ribosome the sequence of the amino acids needed to make the protein.
- They participate in fatty acid metabolism.

5. A microbody is a cytoplasmic organelle of a more or less globular shape that comprises degradative enzymes bound within a single membrane. Microbodies are specialized as containers for metabolic activity. Microbodies are roughly spherical in shape, bound by a single membrane, and are usually 0.5 to 1 micrometre in diameter. There are several types, by far the most common of which is the peroxisome. Peroxisomes derive their name from hydrogen peroxide, a reactive intermediate in the process of molecular breakdown that occurs in the microbody. Peroxisomes contain type II oxidases, which are enzymes that use molecular oxygen in reactions to oxidize organic molecules. A product of these reactions is hydrogen peroxide, which is further metabolized into water and oxygen by the enzyme catalase, a predominant constituent of peroxisomes.

Types

Types include peroxisomes, glyoxysomes, glycosomes and Woronin bodies.

Functions

- Glyoxysomes are found in germinating seeds of plants as well as in filamentous fungi. Glyoxysomes are peroxisomes with additional function - glyoxylate cycle.
- Glycosomes, besides peroxisomal enzymes, also possess glycolysis enzymes and are found in kinetoplastida like Trypanosomes.
Woronin bodies are special organelles found only in filamentous fungi. One established function of Woronin bodies is the plugging of the septal pores after hyphal wounding, which restricts the loss of cytoplasm to the sites of injury.

Functions of peroxisomes

- Oxidation of cellular substrates, such as amino acids, fatty acids, lactic acids, uric acids, purines etc.
- Breakdown of harmful products, such as ethyl alcohol, methyl alcohol, formic acid, nitrates, etc.
- Bio synthesis of phospholipids and the production of plasmalogens.
- Thermogenesis in animals and photo respiration in plants.

6. Nucleus

Nucleus was first discovered by Robert Brown (1831) in orchid cells. It is the most important part of the cell which directs and controls all the cellular functions. That’s why nucleus is very often regarded as ‘director of the cell’. Presence of true nucleus with nuclear membrane and linear chromosomes is the characteristic of all the eukaryotic cells. However, there are some exceptions viz., mature mammalian RBCs, sieve tubes of phloem, tracheids and vessels of xylem.

As far the number of nucleus in a cell is concerned, most of eukaryotic cells have single nucleus within them. However, the number may vary in some cells. The fluid in which nucleolus and chromosomes are present and which is enclosed in nuclear membrane is called nucleoplasm.

A nucleus may be described as having three important parts, viz

Nuclear membrane or nuclear envelope

Nucleolus

Chromosomes

Depending on the number of nuclei cells may be of following types:

- Anucleate (without nucleus) : Mammalian RBCs.
- Uninuclate : Most of Eukaryotic Cells.
- Binucleate : Basidiomycetes, Paramoecium
- Multinucleate : Phycomycetes like Mucor, Rhizopus etc., Red Algae.

Composition:

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protein:70%, DNA: 10%, phospholipid: 3-5%, RNA, RNA primer, chromatin, histones, Ca++, Mg++, K+, P, Fe, N

1. **Nuclear Membrane**: It limits the nucleus externally and also known as **karyothica**. It is bilayedred, lipoproteineous and trilaminar in nature. Outer membrane is called **ectokaryotheca** and the inner is **endokaryotheca**. The outer membrane is studded with ribosomes while the inner is free of that. The two membranes have a thickness of 75-90 Å

2. **Nucleoplasm**: It is transparent semi fluid, homogenous, colloidal ground substance inside the nuclear membrane. It is also called nuclear sap, karyolympf or **karyoplasm**. Nuclear chromatin and nucleolus are embedded within nucleoplasm, chemically, it is formed of water, sugars, minerals (Mn²⁺, Mg²⁺, etc.), Nucleotides, ribosomes, enzymes, DNA and RNA polymerases, mRNA, tRNA molecules etc. It is alkaline in nature (pH = 7.4).

3. **Chromatin Net or thread**: Electron microscopic studies of well stained eukaryotic nuclei have revealed that presence of darkly stained network of long, fine and interwoven threads which is called chromatin net or thread. It is also known as nuclear reticulatum. It was first reported by Fleming in 1882. During the phase of cell division, the chromatin net is transformed into chromosomes due to high condensation of DNA molecules. These chromosomes are rod like and have definite shape and size characteristic of an organism.

4. **Nucleolus**: Within each nucleus, there is a darkly stained, granular, naked and large organelle without limiting membrane. It was discovered by Fontana in 1781. The term nucleolus was coined by Bowman (1840). The size of nucleolus is comparatively larger in those cells which have rapid rates of protein biosynthesis.

The position of nucleolus is generally definite within nucleus.

**Functions of Nucleus**

- It controls all the cellular functions.
- It controls the synthesis of all the structural and enzymatic proteins.
- Synthesis of all the 3 types of RNA (mRNA, tRNA and rRNA) takes place in the nucleus.
- It plays important role in cell division.
- Cell growth is controlled by nucleus
- Nucleus controls cellular differentiation by regulating differential gene expression
- It induces genetic variation and thus helps in organic evolution.
- Sexual reproduction happens due to fusion of two nuclei gametes of opposite sex.

Due to presence of all these organelles and other structures, a cell functions as self-regulatory systems and provides a definite set of characteristics to different organisms.
3. Bio physical phenomenon
3.1. The law of thermodynamics
3.2. Diffusion and osmosis
3.3. Concept of water potential

The search of energy is the main theme in the life. Energy is required to do work. We all know that the Sun is the ultimate source of energy. Green plants harvest the energy and convert it to chemical energy. This chemical energy is stored in ATP, and is released when required for biological reactions. The biological reactions are different from chemical reactions as they take place inside the cell at normal temperature and pressure. In order to make it possible, some proteins act as catalysts for these reactions.

ENERGY FLOW

Energy is defined as the ability to do work. It exits in many forms, at many levels, and it is on the move. It enables and it destroys as it flows in countless directions in the universe. It can be trapped and stored and released to do work at some later time.

**Principles of thermodynamics**

Living organisms also follow principles of thermodynamics i.e. movement of energy. And it’s transformations.

According to the first law of thermodynamics “energy can be neither created nor destroyed, but only changed from one form to another. Living organisms thus cannot consume or use up energy; they can only transform one form of energy into another”.

The second law of thermodynamics tells us that “in any energy transformation, not all energy is transformed, and some energy will be lost simply because no transformations are 100% efficient. It also states that all physical and chemical processes always precede with an increase in the disorder or randomness of in the world i.e. its entropy”. Entropy is a measure of disorder, which means that as the available energy decreases in a system, the system becomes increasingly disorganized. Energy, then, must be added to a system to keep it in an original state.

Third law of thermodynamics: **The entropy of a system approaches a constant value as the temperature approaches zero.** The entropy of a system at absolute zero is typically zero, and in all cases is determined only by the number of different ground states it has.

**Some terminologies:**

**System**: system is a set of interacting or interdependent components forming an integrated whole or a set of elements (often called 'components') and relationships which are different from relationships of the set or its elements to other elements or sets.

**Surroundings** In thermodynamics, the term (and its synonym, environment) is used in a more restricted sense, meaning everything outside the thermodynamic system. Often, the simplifying
assumptions are that energy and matter may move freely within the surroundings, and that the surroundings have a uniform composition.

**Universe**: the system and surrounding together constitute the universe.

**Boundary**: the system is separable from the surrounding by a boundary which may be real or imaginary.

**Homogenous system**: the system is completely uniform throughout

**Heterogeneous system**: the system is not uniform throughout.

**Open system**: An open system is a system which continuously interacts with its environment. The interaction can take the form of information, energy, or material transfers into or out of the system boundary, depending on the discipline which defines the concept.

**Closed system**: In thermodynamics, a closed system can exchange energy (as heat or work), but not matter, with its surroundings. In contrast, an isolated system cannot exchange any of heat, work, or matter with the surroundings, while an open system can exchange all of heat, work and matter.

Enthalpy: **Enthalpy** is a measure of the total energy of a thermodynamic system. It includes the internal energy, which is the energy required to create a system, and the amount of energy required to make room for it by displacing its environment and establishing its volume and pressure.

\[ H = U + pV \]

Where: \( H \) is the enthalpy of the system, \( U \) is the internal energy of the system, \( p \) is the pressure of the system, \( V \) is the volume of the system.

**Entropy**: a quantitative measure of the amount of thermal energy not available to do work. It is a measure of the disorder or randomness in a closed system.

Entropy=unavailable energy/temperature

**Endothermic**: In thermodynamics, the word **endothermic** ("within-heating") describes a process or reaction in which the system absorbs energy from the surroundings in the form of heat. It is a modern coinage formed from Greek roots. The prefix *endo-* derives from the Greek word *endon* (ἐνδόν) meaning "within," and the latter part of the word comes from the Greek word root "therm" (θερμ-) meaning "hot." Hence it refers to a reaction that needs heat i.e. the heat is absorbed.

**Exothermic**: In thermodynamics, the term **exothermic** ("outside heating") describes a process or reaction that releases energy from the system. Its etymology stems from the prefix *exo* (derived from the Greek word ἐξ ὀς, ἐξό, "outside") and the Greek word *thermasi* (meaning "to heat"). The term exothermic was first coined by Marcellin Berthelot. The concept is frequently

---prepared by: Subodh Khanal
applied in the physical sciences to chemical reactions, where chemical bond energy is converted to thermal energy (heat).

**Exergonic** (from the suffix exo-, derived from the Greek word ἔξω exō, "outside" and the suffix -ergonic, derived from the Greek word ἔργον ergon, "work") means "releasing energy in the form of work". In thermodynamics, work is defined as the energy moving from the system (the internal region) to the surroundings (the external region) during a given process. An exergonic process is one in which there is a positive flow of energy from the system to the surroundings. Constant pressure, constant temperature reactions are exergonic if the Gibbs free energy is negative (ΔG < 0).

**Endergonic** (from the prefix endo-, derived from the Greek word ἐνδόν endon, "within", and the Greek word ἔργον ergon, "work") means "absorbing energy in the form of work." An endergonic process, as contrasted with an exergonic process, is one wherein the system absorbs energy from the surroundings. As a result, during an endergonic process, energy is put into the system. If the transformation occurs at constant pressure and temperature, ΔG > 0. An endergonic reaction is a chemical reaction that absorbs energy in the form of work. A good example of a net endergonic process is photosynthesis. Also, in metabolism, an endergonic process is anabolic, meaning, that energy is stored. In metabolism, catabolic and anabolic processes are coupled by ATP.

**Gibbs free energy**

In thermodynamics, the **Gibbs free energy** (IUPAC recommended name: Gibbs energy or Gibbs function; also known as free enthalpy) is a thermodynamic potential that measures the "useful" or process-initiating work obtainable from a thermodynamic system at a constant temperature and pressure (isothermal, isobaric).

**Definitions**

The Gibbs free energy is defined as:

\[ G(p,T) = U + pV - TS \]

which is the same as:

\[ G(p,T) = H - TS \]

where: \( U \) is the internal energy (SI unit: joule), \( p \) is pressure (SI unit: pascal), \( V \) is volume (SI unit: m\(^3\)), \( T \) is the temperature (SI unit: kelvin), \( S \) is the entropy (SI unit: joule per kelvin), \( H \) is the enthalpy (SI unit: joule)

The Gibbs free energy of a system at any moment in time is defined as the enthalpy of the system minus the product of the temperature times the entropy of the system.

\[ G = H - TS \]
The Gibbs free energy of the system is a state function because it is defined in terms of thermodynamic properties that are state functions. The change in the Gibbs free energy of the system that occurs during a reaction is therefore equal to the change in the enthalpy of the system minus the change in the product of the temperature times the entropy of the system.

\[ \Delta G = \Delta H - \Delta (TS) \]

If the reaction is run at constant temperature, this equation can be written as follows.

\[ \Delta G = \Delta H - T \Delta S \]

The change in the free energy of a system that occurs during a reaction can be measured under any set of conditions. If the data are collected under standard-state conditions, the result is the standard-state free energy of reaction (\( \Delta G^0 \)).

\[ \Delta G^0 = \Delta H^0 - T \Delta S^0 \]

**Isothermal process** is a change of a system, in which the temperature remains constant: \( \Delta T = 0 \). This typically occurs when a system is in contact with an outside thermal reservoir (heat bath), and the change occurs slowly enough to allow the system to continually adjust to the temperature of the reservoir through heat exchange. In contrast, an **adiabatic process** is where a system exchanges no heat with its surroundings (\( Q = 0 \)). In other words, in an isothermal process, the value \( \Delta T = 0 \) but \( Q \neq 0 \), while in an adiabatic process, \( \Delta T \neq 0 \) but \( Q = 0 \). These laws of energy have some interesting implications for living systems. As living systems, are highly organized, they need constant energy to remain organized. So, these systems must have both a source of energy and some means of converting the energy from one form of energy into another.

The ultimate source of all energy is the Sun; this energy is captured, changed, shifted about and filtered through living systems, where it is used to reduce entropy.

**Application of 1st law of thermodynamics:**

- A living system is an open system where the matter and energy both are exchanged. Some of this energy is used in useful work and some is returned to the surrounding as heat to produce molecules.
- Leaves absorb energy from surrounding in two ways; direct incident radiation from the sun and infrared radiation from the surrounding. Some of the energy is returned back as infrared radiation and rest is used in photosynthesis i.e. energy stored in leaf=energy emitted by leaf+energy stored by leaf
- Sunlight is ultimate source of energy. thermonuclear reaction in the sun produces energy that is transmitted to earth as light and converted into chemical energy by plant and certain microorganisms
- ATP is the universal carrier of metabolic energy linking catabolism and anabolism.
- Potential energy is storage energy present in the body at rest. While kinetic energy of motion that moves a body at the cost of potential energy.
Application of 2\textsuperscript{nd} law of thermodynamics:

- An energy is transformed from one organism to another (plant to herbivores to carnivores and so on). In this process large part of energy is lost as heat.
- In photosynthetic pigment system (PS1 and PS2) both exothermic and endothermic reaction is required.
- The $G=H-TS$. When a chemical reaction occurs at constant temperature, the free energy is determined by ▲H reflecting the kind and number of chemical bond and non constant interaction broken and formed.
- In living system there is anabolic and catabolic process. In anabolism there is less entropy whereas catabolism has higher entropy.

Significance of entropy:

- Entropy is the degree of randomness which is directly proportional to the disorder of system.
- It is the function of thermodynamic probability. Catabolism has higher entropy than anabolism.

3.2. Concept of water potential, diffusion and osmosis

Water has a tendency to move from the region of higher chemical potential to lower chemical potential of water, if the two regions are not separated by a barrier. This movement takes place until there is equilibrium in the two regions. The potential is known as water potential ($\psi$) (psi) and is measured in terms of pressures eg. Bars or atmospheres. One bar = 14.5 lb/m\textsuperscript{2}, 750 nm Hg or 0.987 atm.

The value of water potential for pure water is 0. If a solute is dissolved in it, the water potential will become negative. In case of external pressure water potential will become positive.

Water potential is a diagnostic tool to know a precise value to the water status in the plant cell and tissues. The lower the water potential in a cell, the greater is its ability to absorb water. Similarly, the cell with higher water potential is able to supply water to other cells.

If the two cells are separated, having water potential $\psi_A$ and $\psi_B$, the difference in water potential will be:

$$\Delta\psi = \psi_A - \psi_B$$

When a typical plant cell containing cell-wall, vacuole and cytoplasm is subjected to movement of water, a number of factors determine the water potential of the cell-sap. The water potential of a living cell is determined by three major potentials. – (1) matric potential ($\psi_m$), (2) solute potential ($\psi_s$) and (3) pressure potential ($\psi_p$). The water potential ($\psi$) is

$$\psi = \psi_m + \psi_s + \psi_p$$
(1) Matric potential ($\psi_m$) - Matric are surfaces which can absorb water molecules, eg., cell-walls, protoplasm and soil particles etc. Matric potential ($\psi_m$) is the component of water potential influenced by the presence of a matric and posses negative value. The value of $\psi_m$ is negligible in case of plant tissues and is insignificant in osmosis. The equation is therefore may be-

$$\psi = \psi_s + \psi_p$$

(2) Solute potential ($\psi_s$) – it is the amount of solute present in water and also known as osmotic potential. The presence of solute in water reduces the value of water potential and hence the value of water potential goes the negative.

(3) Pressure potential ($\psi_p$) – the plant cell wall provides a definite shape to the cell and is elastic in nature. It also exerts a pressure on the cellular contents inwards resulting into development of hydrostatic pressure in the vacuole. The values of $\psi_p$ in plant cells are positive.

Water potential of protoplasm is equal but opposite to that of diffusion pressure and suction pressure. i.e.  $\psi$= - DPD

**Importance of water potential:**

- It is an important force which determines the water status of plant cell or organ.
- The osmotic movement of water out of cell is due to water potential.
- The cell suffering from water deficit is therefore able to avoid injury by obtaining water from adjacent cell.
- The air dried seeds and spores are able to avoid stress and injury due to very low water potential.

Osmotic relation between solute potential($\psi_s$), pressure potential ($\psi_p$) and water potential ($\psi$)

- In fully turgid cell, ($\psi$) =0
- In flaccid cell, $\psi_s$=$\psi$
- In plasmolyzed cell $\psi$ is more negative

**Diffusion**

Diffusion refers to the process by which molecules intermingle as a result of their kinetic energy of random motion. The tendency toward diffusion is very strong even at room temperature because of the high molecular velocities associated with the thermal energy of the particles.

**Rate of Diffusion**

Since the average kinetic energy of different types of molecules (different masses) which are at thermal equilibrium is the same, then their average velocities are different. Their average
diffusion rate is expected to depend upon that average velocity, which gives a relative diffusion rate

\[
\text{diffusion rate} = K \sqrt{\frac{T}{m}}
\]

where the constant K depends upon geometric factors including the area across which the diffusion is occurring. The relative diffusion rate for two different molecular species is then given by

\[
\frac{\text{diffusion rate of } A}{\text{diffusion rate of } B} = \sqrt{\frac{m_B}{m_A}}
\]

DPD of a solution is equal to its osmotic pressure i.e. DPD = OP (of solution). DPD of a cell is influence by both osmotic pressure and wall pressure (turgor pressure) which opposes the endosmotic entry of water, i.e. DPD = OP - Wall presseure. DPD is directly proportional to the concentration of the solution. DPD decreases with dilution of solution. The actual pressure with which cell absorbs water is called "suction pressure". In refrigeration and air conditioning systems, the suction pressure' (also called the low-side pressure) is the intake pressure generated by the system compressor while operating. The suction pressure, along with the suction temperature and the wet bulb temperature of the discharge air are used to determine the correct refrigerant charge in a system. If some solute is dissolved in water its diffusion pressure decreases. The difference between diffusion pressure of pure water and solution is called diffusion pressure deficit (DPD).

When a plant cell is placed in hypotonic solution, water enters into a cell by osmosis and as a result turgor pressure develops. The cell membrane get stretched and osmotic pressure of cell decreases. As cell absorbs more and more water its TP increases and OP decreases. When a cell is fully turgid, its OP is equal to TP and DPD is zero. Turgis cell cannot absorb any more water. Thus, with reference to plant cell, the DPD can be described as actual thirst of cell for water and can be expressed as DPD=OP-TP.

When DPD is zero, entry of water will stop. Thus it is DPD that tends to equate and represents water absorbing ability of a cell, it is also called suction force (SF)or suction pressure(SP).

**Factors Affecting Rate of Diffusion**

Factors Pertaining to the solution or Molecule

1) Temperature

→ An increase in temperature increases the rate of diffusion.

→ Molecules gain more energy therefore move faster which increases rate of diffusion, KMT.

2) Concentration

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The greater the difference in concentration between two regions of a substance the greater the rate of diffusion.

An increase in concentration increases the pressure of the molecules to diffuse into the area of low concentration. (more particles trying to get to the same area at the same time)

3) Size and nature of the molecule
   → Small molecules diffuse faster than large ones.
   → Fat-soluble (non-polar) molecules diffuse faster through the non-polar membrane than polar or water soluble ones.

Factors Pertaining to the Cell

4) Cell structure
   A) The shorter the distance between the two regions (smaller volume) the faster the diffusion.
   B) The larger the surface area for diffusion the faster the rate (more molecules are able to pass through at the same time).
   C) Variations in membrane structure may affect diffusion (i.e. the greater the number and size of pores in the membrane the greater the rate of diffusion).

Significance of diffusion in plants:

i. It is an essential process in exchange of gases (O2 and CO2) during respiration and photosynthesis.
ii. Uptake of minerals is also affected by the process of diffusion.
iii. It helps in removal of excess water by the process of transpiration.
iv. Translocation of organic solutes also takes place by diffusion means.
v. Fragrance of flowers or sweet scent emitted by flowers spread in air by diffusion means attracts insects to materialize pollination.

Osmosis

When two solutions of different concentrations are separated by a differentially permeable membrane, the diffusion of water or solvent molecules is from their higher concentration to their lower concentration. This process is called osmosis. Actually the movement of solvent molecules takes place across the membrane on both the sides but it is faster from the higher to lower concentration. From the biological point of view the solutions are of three types-

1. Hypertonic solutions- solutions whose concentration and osmotic pressure are more than the concentration and osmotic concentration of cell sap.

2. Hypotonic solutions- solutions whose concentration and osmotic pressure are less than the concentration and osmotic concentration of cell sap

3. Isotonic solutions- solutions whose concentration and osmotic pressure are equal to the concentration and osmotic concentration of cell sap.
Types of Osmosis –

1. Endosmosis- When water or solvent molecules enter into the cell through the plasma membrane from the outer medium, it is called endosmosis. Eg. When dry raisins are placed in the water, they swell up due to endosmosis.

2. Exosmosis- When a cell is placed in concentrated solution, the water or solvent molecules move from the outer concentrated solution through the plasma membrane. it is called exosmosis. Eg. When grapes are placed in the concentrated sugar solution, they shrink due to exosmosis. Such solutions are Hypertonic solutions- solutions whose concentration and osmotic pressure are more than the concentration and osmotic concentration of cell sap.

Osmotic pressure is the pressure which needs to be applied to a solution to prevent the inward flow of water across a semi-permeable membrane. It is also defined as the minimum pressure needed to nullify osmosis.

The phenomenon of osmotic pressure arises from the tendency of a pure solvent to move through a semi-permeable membrane and into a solution containing a solute to which the membrane is impermeable. This process is of vital importance in biology as the cell's membrane is selective toward many of the solutes found in living organisms.

Applications

Osmosis is of immense importance to plants.

i. large quantities of water are absorbed by root and root hairs from soil by osmosis.

ii. The movement and distribution of water across the cells of the plant takes place by the process of osmosis.

iii. Endosmosis and exosmosis arc responsible for opening and closing of the stomata.

iv. The form and shape of many cells and the keeping of the plasma membrane near the cell wall are dependent upon osmotic phenomena.

v. High osmotic concentration increases the resistance of plants to freezing temperature and desiccation.

vi. The turgor of guard cells is absolutely essential for opening of stomata.

vii. In lover plants turgor pressure provides mechanical strength.

viii. Growth of young cells is brought about by the osmotic pressure and turgor pressure of these cells.

ix. Many plant movements involve changes in turgor which are brought about by osmosis.

x. The various cell organ cells such as chloroplasts, mitochondria etc. will collapse if they are not able to maintain a proper osmotic concentration of solutes.
4. Adsorption and translocation of water and minerals
4.1. Adsorption of water, mechanisms and factors
4.2. Ascent of sap: concept and mechanism
4.3. Mineral absorption and translocation
4.4. Transpiration: concept, type, importance and mechanism

The ancestors of modern day plants evolved in water and it is no surprise that the internal environment of a plant is 80-90% water. The relative humidity inside plants is always higher than 95% and usually higher than 98%. As land plants must remain hydrated to this level if they are to continue growing, they have evolved a number of mechanisms, such as waxy cuticles, to ensure that they do not dry out.

The plant can control the loss of water from its leaves by varying the aperture of its stomata (like a tap). However, if a plant restricts the flow of water vapour out of its leaf it automatically restricts the flow of CO₂ into the leaf for photosynthesis. This is known as the transpiration compromise or the plant version of 'there are no free lunches'. On average, for each 100 litres of water used by a plant, each process uses:

Photosynthesis 0.1 litres
Growth (new leaves, roots etc) 1.9 litres
Transpiration 98 litres

Absorption of water

The absorption of the water in different types of plants is performed by special organs. In most of the plants, the roots absorb the water from the soil. A root possesses following regions-

1. Root cap zone
2. Meristematic zone
3. Zone of elongation
4. Zone of root hair
5. Mature Zone.

Internal structure of root shows a clear channel for water movement as depicted in the figure-

Movement of Water Through Cells - Two Routes, the Symplast and the Apoplast

Symplastic Movement
• Movement of water and solutes through the continuous connection of cytoplasm (though plasmodesmata)

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• No crossing of the plasma membrane (once it is in the symplast—however, if the solute was initially external to the cell, then it must have crossed one plasma membrane to enter the symplast)

**Apoplastic Movement**
• Movement of water and solutes through the cell walls and the intercellular spaces
• No crossing of the plasma membrane
• More rapid - less resistance to the flow of water

Water in the roots move by two pathways. They can be classified as 1) Apoplast pathway 2) Symplast pathway
MECHANISM OF WATER ABSORPTION-

Soil is made up of irregularly arranged soil particles which have well developed air spaces between them. The spaces contain capillary water where air and mineral salts remain dissolved. The root hairs remain in contact with this water. The mechanism of water absorption is of following two types-

1. **Active absorption**- When roots are actively involved in water absorption, water absorbing forces develop in the roots. When transpiration is less and water is present in sufficient amounts. Active absorption requires ATP released during respiration. It is also of two types:

   (a) **Osmotic absorption**- In this type of water absorption, the roots act like an osmometer and water is absorbed according to osmotic gradient. As the cell wall of root hair is permeable and allows entry and exit of the liquids through it. Plasma membrane is differentially permeable and allows only the diffusion of water and important dissolved salts into the cytoplasm. The cytoplasm of root hair is usually concentrated than the capillary water. The difference between the water potential between the two results in endosmosis of water and dissolved substances into the root hair.

   After sometime the cells of root hairs become turgid and their water potential will increase in comparison to the cytoplasm of the adjacent cells resulting in the osmotic diffusion of water and salts from root hair cell to the first cortical cell. This process continues as follows:
   
   Root hair $\rightarrow$ Epidermal cell $\rightarrow$ various successive cortex cells $\rightarrow$ Endodermal cells $\rightarrow$ Cells of pericycle $\rightarrow$ Xylem cells $\rightarrow$ Xylem duct $\rightarrow$ Upward movement of water.

   **Root pressure**- When the water enters into xylem vessels, a pressure is created in the xylem of roots due to which the water rises to a certain height in the xylem. This pressure is root pressure.

   (b) **Non-osmotic absorption**- In this type of water absorption, more ATP is required and the water absorption takes place against osmotic gradient. When the water potential of soil water, is greater than that of cytoplasm of root hair, the movement of water molecules is against the osmosis, so, energy is required in the form of ATP. The energy may be utilized directly or indirectly.

   The non-osmotic active water uptake can also be supported by the following facts:
   (i) There is a correlation between the rate of water absorption and respiration,
   (ii) Auxin-induced water uptake
   (iii) The factors such as low temperature, oxygen tension and respiratory inhibitors which affect respiratory rate and in turn the absorption rate.

The serious drawback of the above theory is that it fails to explain the exact manner of utilization of metabolic energy in the process of water uptake.

2. **Passive absorption** - When roots are inactive in water absorption, and water absorbing forces develop primarily in the leaves and stem and then reach to root through xylem, this type of water absorption is called passive absorption. It takes place mainly due to transpiration, when water diffuses from the xylem cells of leaves to all mesophyll cells. When the rate of transpiration is

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high, a tension is created in the water column of xylem and it decreases the water potential of them. This tension moves from leaves to roots and makes the root hair cells to fetch water from surrounding soil. Passive absorption is found when transpiration is very fast and soil contains abundant water. No ATP is required for this absorption.

### Comparison chart

<table>
<thead>
<tr>
<th>Active absorption</th>
<th>Passive absorption</th>
</tr>
</thead>
<tbody>
<tr>
<td>The active absorption of water takes place due to the activity of root and root hairs.</td>
<td>It takes place due to activity of upper of the plant such as shoot and leaves.</td>
</tr>
<tr>
<td>The absorption of water occurs by osmotic and non osmotic process.</td>
<td>The water is absorbed due to the action transpiration in aerial parts.</td>
</tr>
<tr>
<td>Involves the expenditures of metabolic energy</td>
<td>Does not involve the expenditure of energy.</td>
</tr>
<tr>
<td>Occurs either along or against potential gradient.</td>
<td>Occurs along a osmotic potential gradient.</td>
</tr>
<tr>
<td>In the movement of water, living parts of protoplast (symplast) is involved.</td>
<td>The movement of water is through apoplast of root.</td>
</tr>
<tr>
<td>It creates positive pressure in xylem sap.</td>
<td>It creates negative pressure in xylem sap</td>
</tr>
<tr>
<td>The absorption of water occurs by secretion, electro-osmotic, osmotic and non osmotic mechanism</td>
<td>The absorption occurs by apoplast, transmembrane.</td>
</tr>
<tr>
<td>It can meet only 4-5% of water requirement of plants.</td>
<td>It meet all the requirement under favorable condition.</td>
</tr>
<tr>
<td>The rate of water absorption is maximum during night or early morning.</td>
<td>The rate of absorption is maximum during noon.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Concentration Gradient:</th>
<th>Active Transport</th>
<th>Passive Transport</th>
</tr>
</thead>
<tbody>
<tr>
<td>Requires Energy:</td>
<td>Against</td>
<td>With</td>
</tr>
<tr>
<td>Types of Transport:</td>
<td>Yes; ATP in primary, electrochemical in secondary.</td>
<td>No</td>
</tr>
<tr>
<td>Types of Particle:</td>
<td>Primary and secondary</td>
<td>Diffusion, facilitated diffusion, filtration, osmosis.</td>
</tr>
<tr>
<td>Examples:</td>
<td>Endocytosis; Exocytosis; membrane pumps;Sodium pump; glucose in intestines; mineral ions in plant roots</td>
<td>diffusion; osmosis; facilitated diffusion;Alveoli of lungs; kidneys</td>
</tr>
</tbody>
</table>

**Importance:**

- Active cell stage is due to 90-95% water.
- Water participates directly in metabolism

---prepared by: Subodh Khanal
- Most common solvent and carries the nutrients.
- Supplies reduction power in oxidation reduction reactions.
- Maintain the structure of metabolites.
- Thermoregulation in plants.
- Size and shape of cells are maintained.
- Helps in cellular movement and translocation of nutrients.

**ASCENT OF SAP**

The water and soluble mineral salts absorbed by the roots reach to the leaves through roots, stem and branches of the plant. The phenomenon of ascending of absorbed water against gravitational force through the vessels and tracheids of xylem is called **ascent of sap**.

**Path of ascent of sap or mechanism of water transport through xylem**-

You must have studied about the experiments like ringing experiments which clearly showed that the water and dissolved salts ascend in the stem through vessels and tracheids of xylem tissue. Now, the question arises, how the water arises up to as high as 115 meters? Various theories have been given to understand this phenomenon-

These theories can be divided into two groups:
(a) Vital theories, and  (b) Physical theories.

**Vital theories**

1. **Root pressure theory**- The plants absorb water from soil through root hair. The inflow of water exerts pressure on xylem cells and this pressure pushes the water in upward direction –this pressure is known as root pressure.

   But the root pressure is of only 2 atmospheres which helps the water to ascend up to a maximum height of 20 meters. Similarly if we cut root system of a plant and put it vertically in water even then the upward movement of water does not stop. Thus, the root pressure theory proves to be wrong.

2. **Godlewski theory**- Godlewski (1884) proposed that the water ascends in the plant due to pumping action of the cells of xylem parenchyma and medullary rays.

3. **Vital force theory**- Sir J.C. Bose (1923) suggested that the cells of inner layer of cortex pulsating action like heart. When these cells expand, the water from adjacent cortical cells diffuses into them and they become turgid. When these cells contract, the water ascends in the plant forcibly.

   Strasburger (1893) proved all the vital theories wrong. He observed the continuous ascending movement of water even if the cells of parenchyma and medullary rays were destroyed with the help of poisons like picric acid.

**Physical Theories**-

1. **Imbibition theory**- It was proposed by Unger (1868). The theory says that the water ascends due to imbibitional forces through the walls of xylem tracheids and vessels and their lumen and cavities have no relation with the ascent of sap, but now it has been proved that the ascent of sap stops if the lumens of xylem vessels and tracheids are blocked with the wax. Thus, the imbibition theory is also proved to be wrong.
2. Capillary force theory- It was proposed by Boehm (1899). He said that the water ascends in the xylem vessels through capillary action. It is similar to the rising of water in a fine capillary. It has been proved that through capillary force the water can rise only up to a small distance. Thus, the theory is also wrong and will not be applicable to very tall plants.

3. Cohesion theory of Dixon and Jolly- It was proposed by Dixon and Jolly (1894). It is most widely accepted view. They explained that the water molecules are attracted to each other by a force called cohesive force. The cohesive force maintains the continuity of water column in the xylem vessels and a continuous water column is formed roots to leaves. The water evaporates continuously from the leaves due to transpiration. The mesophyll cells of leaves possess intercellular spaces filled with water vapors. The stomata are connected with these spaces. The loss of water in these spaces is compensated by mesophyll cells which gets the water from the adjacent cells. This process continues slowly up to the cells of xylem vessels. The xylem vessels of leaves are connected with xylem vessels of stem and roots. Due to this cohesive force of water molecules, the water ascends to a great height. This pulling force or suction force is called transpiration pull and the water column in the xylem vessels as transpiration stream because the force is created due to transpiration. The cohesive force of water is as high as 350 atmospheres.

Objection:
- Transpiration is not solely responsible for upward tension in the water column.
- Air vessels are frequently found in the vessels and trachids that may break the continuity of water column.
- Water column break down even on herbaceous plants.
- High wind velocity, high variation of temperature may cause the braking of continuity of water column.

Evidences in support
- Osmotic potential of mesophyll have reached up to 200atm.
• Purely physical process
• The rate of water absorption depends upon the rise and fall in the rate of transpiration.
• The tensile strength of the xylem sap is in between 25-300 atm which is sufficient enough to maintain a continuous water column.

Mineral salt absorption
Earlier it was thought that inorganic salts are passively carried into plants with the absorption of water, and the absorbed salts are translocated to the different parts of the plant through transpiration stream. The osmotically active solutes were supposed to diffuse from soil solution into the plant along the concentration gradient. But, now it has been established that mineral salt absorption is an active process rather than a passive process. The mineral salt absorption may be of two types – Passive absorption and Active absorption

Passive absorption
When the absorption of mineral salts takes place without any expenditure of metabolic energy (ATP) and simply by diffusion into the plant cells, it is called passive absorption.

1. Outer and apparent free space theory-
Salt absorption takes place through the intimate contact of the root system with the soil solutions. The root system is made up of cells. All the cells when immersed in the salt solution are open to free diffusion of ions. Here, the ions can move freely in or out of the tissue, until they reach equilibrium with the external medium. The part of the plant cell or tissue which allows diffusion is called an outer space

2. Ion Exchange –
The mineral elements are absorbed by plants in the form of ions. The anions or cations of the plant cells are exchanged from the anions or cations of the equivalent charge from the external medium. The ion exchange mechanism has been explained by following two theories-

a) The contact exchange theory-
Jenny and Overstreet (1939) proposed that the ions are transferred from soil particles to the root or vice versa without passing through the free solutions. The ions are adsorbed electrostatically to the surface of the root cells or clay particles, they are not firmly held. Due to thermal agitation each of them oscillates within a small volume of the space- known as oscillation volume. When the oscillation volumes of two ions with same charge overlap, one ion is exchanged from the other. This process has been called contact exchange.

---prepared by: Subodh Khanal
b) Carbonic acid exchange theory
According to this theory the soil solution plays an important role. The CO$_2$ released during respiration of root cells combines with water to form carbonic acid (H$_2$CO$_3$) in the soil solution. Carbonic acid dissociates into H$^+$ and HCO$_3^-$. A cation for e.g. K$^+$ absorbed on the clay micelle may be exchanged with H$^+$ of the soil solution. This cation K$^+$ may diffuse to the root surface in exchange for H$^+$ ions. The cations may also be absorbed on root cells in exchange as ion pairs with bicarbonate.

3. Donnan equilibrium
In Donnan equilibrium the fixed or indiffusible ions play an important role. (Donnan, 1927). As per this theory there are certain pre-existing ions inside the cells which can not diffuse outside through membrane. Such ions are called fixed or indiffusible ions. The outer membrane is impermeable to fixed ions. However, the cell membrane is permeable to both anions and cations present in the external medium. When a cell is put in an external salt solution and on the inner side of there are fixed anions, the movement of equal number of anions and cations will take place. In addition to this additional cations will also come to balance the negative charges of the fixed ions. This balance or equilibrium is called Donnan equilibrium. Here, the cation conc. in the cell-sap would be greater than in the external medium.

Similarly, if the membrane is impermeable to fixed cations, additional anions will accumulate from the external medium.

The Donnan equilibrium theory explains the accumulation of ions against a concentration gradient without the participation of metabolic energy.

4. Mass flow-
Hylmo (1953) believed that ions can move through roots along with the mass flow of water. An increase in transpiration increases the absorption of ions.

Theories of Salt absorption-
Based on carrier concept there are two possible mechanisms for salt absorption.
1. Cytochrome pump theory
2. Carrier Mechanism Involving ATP

According to carrier concept
• The proteins may assist in the movement of substances by facilitated diffusion or active transport.
• These mechanisms of movement are known as carrier mediated transport.
• Each carrier protein is designed to recognize only one substance or one group of very similar substances.
• Active transport involves the use of an electrochemical gradient, and does not use energy produced in the cell. A carrier protein is required to move particles from areas of low concentration to areas of high concentration. The carrier protein substrate is released at that site, according to its binding affinity there.
• Facilitated diffusion is the passage of molecules or ions across a biological membrane through specific carrier proteins and requires no energy. Facilitated diffusion is used especially in the case of large polar molecules and charged ions; once such ions are dissolved in water they cannot diffuse freely across cell membranes due to the hydrophobic nature of the fatty acid tails of the phospholipids that make up the bilayers.
• The substrate is taken in one side of the gated carrier, and without using ATP the substrate is released into the cell.

In carrier mechanism, activated ions combine with carrier proteins and form ion carrier complex. This complex moves across the membrane and reaches the inner space by the expenditure of energy. Within the cytoplasm, the complex breaks to release the ions. The carrier moves out of the cytoplasm and is again ready to attach another ion to from a complex.

**Cytochrome Pump Salt Respiration or Electron Transport Theory**

This theory was proposed by H. Lundegardh, who suggested that anions could be transported across the membrane by cytochrome system. Energy is supplied by direct oxidation of respiratory intermediates.
Diagrammatic representation of cytochrome pump hypothesis: On salt absorption, anions ($A^-$) are actively absorbed via a cytochrome pump and cations ($M^+$) are passively absorbed.

The rate of respiration, which is solely due to anion absorption, is called as anion respiration or salt respiration. The original rate of respiration (without anion respiration) can be observed in distilled water and is called ground respiration.

Total respiration ($R_t$) = Ground respiration ($R_g$) + Salt or anion respiration ($R_a$).

**Translocation of nutrients:**
In the translocation process absorbed nutrients have to be distributed throughout the plant body. Initially the nutrients have to be entered through different layers of endodermal cells to reach the xylem duct where accumulation against the concentration gradient by active process takes place.

- Nutrients accumulated in ducts are circulated in the different parts through vascular tissues.
- Translocation in the xylem duct takes place in transpiration stream.
- Lateral movement of salts/nutrients as xylem and phloem tissues are separated by a layer of living cells constituting cambium tissues.
- Downward and outward movement of salts is through phloem.

**General function of essential elements in plants:**
1. Constituent of protoplasm and cell walls: $C$, $H$,$O$, $N$,$P$, $S$ are very important constituent of protoplasm and cell wall. $N$ for protein and nucleic acid, $S$ for protein and $p$ for nucleic acid and most importantly $C$, $H$, $O$ forms most of the plant body.

2. Influence on osmotic pressure of plant cells.
3. Catalytic function: elements like Fe, Cu, Zn, Mo, Mn, and Cl are the part of prosthetic group of enzymes or co enzymes.

4. Antagonistic or balancing function: Ca, mg, K counteracts the toxic effect of other minerals by maintaining ionic balance.

Factors affecting mineral translocation
1. External: light, aeration, temperature
2. Internal: growth, ageing

FACTORS THAT CONTROL ABSORPTION

Soil aeration: Water logged soils or soils with higher content of clay have very little amount of air; under such conditions roots are subjected to anaerobic conditions and the absorption of minerals is drastically affected.

TEMPERATURE
If the temperature of the soil is lowered, absorption of minerals will be drastically reduced; but with the increase in temperature, the rate of absorption also increases, but up to certain limits.

pH OF THE SOIL SOLUTION

The degree of ionization of minerals and other nutrients depends upon the hydrogen ion concentration of the soil solution. On the other hand neutral pH favors the absorption of monovalent ions. So the soil pH has a significant effect not only on the rate but also the kind of ions uptake. Too acidic or too alkaline soil is virtually useless for cultivation. Until and unless the soils are restored in terms of pH, such soils remain as wastelands.

CONCENTRATION OF SOIL SOLUTION

Generally the concentration of minerals and its components found in soil solution is far below the levels of the same found in the cell sap. It means that the absorption of ions takes place against concentration gradient. The relative concentration of ions found in the cell sap and soil solution gives absorption ratio.

4.4. Transpiration

The water absorbed by the roots is not retained by the plant as a whole but only a small amount is utilized by the plant. The excess amount is transpired through the aerial parts of the plant. *The loss of water in the form of vapors from the various aerial parts of the plants is called transpiration.*

KINDS OF TRANSPERSION-

Transpiration may be of following three types-
- Cuticular Transpiration
The loss of water in the form of vapors from the cuticle of various plant parts is called cuticular transpiration. The water vapors reach to the cuticle through the internal tissues of aerial parts by diffusion and from cuticle it diffuses in the atmosphere. Of the total water transpired about 5-10% is lost through cuticle.

- **Lenticular Transpiration**
  The loss of water in the form of vapors through lenticels is called Lenticular transpiration. Lenticels are the small pores present below the bark of old trees. These are meant for exchange of gases but also carry out transpiration.

- **Stomatal Transpiration**
  The loss of water in the form of vapors through the stomata of leaves is called stomatal transpiration. The maximum amount of (80-90%) absorbed water is transpired through stomatal transpiration. It is commonly found in the leaves and stems of young plants.

**STRUCTURE OF STOMATA**

The epidermis of leaves and green stems possess many small pores called stomata. The length and breadth of open stomata is about 10-40 μ and 3-10 μ respectively. Each pore is surrounded by special semi-lunar or kidney shaped living epidermal cells called guard cells. The
pore and guard cells together called stoma (pl. stomata). The stoma opens to the interior into a cavity called sub-stomatal cavity which remains connected with intercellular spaces.

The guard cells possess a nucleus, cytoplasm and several chloroplasts. Their inner walls are thicker than the outer walls. Each guard cell is surrounded by two or more living cells called subsidiary cells or accessory cells.

Distribution of stomata on leaf-
The stomata are found distributed on the upper and lower surfaces of the leaf. The number of stomata in a leaf varies from 1000 to 6000 per square centimeter. All the stomata of a leaf cover about 1-2 % of total area of leaf. Their distribution may be of following 5 types-

A) **Apple type**- When the stomata are found only on the lower surface of the leaf, the condition is called hypostomatous. E.g., Apple, Peach, Mulberry and Walnut.

B) **Potato type**- When the stomata are found more on the lower surface than the upper surface of the leaf. E.g., Potato, Tomato and Pea.

C) **Oat type**- When the stomata are found equally on both the surfaces of the leaf, E.g. Oat, Maize and Grasses.

D) **Water-lily type**- When the stomata are found only on the upper surface of the leaf, E.g. water-lily.

E) **Potamogeton type**- When the stomata are absent or non-functional. E.g. Potamogeton,

**Mechanism of Transpiration**-

The mechanism of transpiration is completed in two stages:
1. The diffusion of water of mesophyll cells into intercellular spaces.
2. The diffusion of water vapours of intercellular spaces into the outer dry atmosphere.
The roots of the plants continuously absorb water and mineral salts from the soil which ascend through the xylem vessels and reach to the leaves. The mesophyll cells of the leaves become turgid due to excess amount of the absorbed water. At this stage the TP of the cell increases and DPD decreases which results in diffusion of water from mesophyll cells into intercellular spaces. The intercellular spaces now become saturated with water and their water vapor pressure becomes greater than the water vapor pressure of atmosphere. Simultaneously, DPD of intercellular spaces becomes much lower than the DPD of water vapors present in the atmosphere. Thus, the water vapors from the intercellular spaces diffuse into the atmosphere in the form of vapors through stomata, lenticels and cuticle.

**Opening and closing of stomata**

The opening and closing of stomata depend upon the turgid and flaccid condition of guard cells. When the guard cells become turgid, stomata open and they close when the guard cells are flaccid. The size of stomatal opening depends upon the degree of turgidity of guard cells. As we know, that the outer wall of guard cells is thin and elastic whereas the inner wall is thicker and non-elastic. When the guard cells absorb water from surrounding cells, they become turgid and their TP also increases. The TP exert a pressure on the outer wall of guard cells resulting in stretching or spreading of the outer walls, but the inner walls are being thick and non-elastic in nature does not spread much. Due to stretching of its outer wall towards outside, the inner wall also stretches outwardly and the stomata opens. When the turgidity and TP of guard cells decreases, both the walls start to come in their original state and the stomata start to close. When the guard cells become flaccid and the TP becomes zero, the outer walls regain their original position and the stomata close.

Different theories were proposed to explain the reasons of change in turgidity and TP of guard cells. These are-

1. **Theory of photosynthesis in guard cells:**

   Von Mohl (1856) observed that the stomata remain open in light or day time and closed during night. So, he proposed that the chloroplast present in guard cells produce carbohydrates by photosynthesis and so the OP of guard cells increases making them turgid. This increased turgidity results in opening of stomata. During night the OP and TP of guard cells are very less or zero due to lack of photosynthesis and the stomata become closed.

2. **Theory of Starch ↔ sugar interconversion:**

   Lloyd, Loftfield and Sayre proposed that the amount of starch in the guard cells increases during night and decreases during day time. The insoluble starch present in the guard cells is hydrolysed into soluble glucose 1-phosphate in presence of enzyme phosphorylase during day time and soluble glucose 1-phosphate is converted into insoluble starch during night.

3. **Theory of Starch ↔ glucose interconversion:**

   The scheme given by Steward (1964) is as follows-

   (A) Opening of stomata:
4. Theory of Glycolate metabolism:
This theory was proposed by Zelitch. He proposed that the glycolic acid plays an important role in the opening of stomata. It is formed in the guard cells when the conc. of CO$_2$ is reduced. Glucolate also synthesizes carbohydrates. The OP of guard cells increases with the formation of glycolate which requires ATP for its synthesis.

5. Theory of Proton Transport:
This theory was proposed by Levitt (1974). According to this theory the opening and closing of stomata depend upon the entry and exit of potassium ions in the guard cells. At first malic acid is formed from starch in the guard cells which dissociates into cations and anions. The anions and exit out from the guard cells and K$^+$ ions come into the guard cells to replace them. The K$^+$ ions react with the malic acid to form potassium malate which is transported into cell vacuoles. It increases the OP of guard cells resulting in diffusion of water from mesophyll cells into guard cells. Thus, the guard cells become turgid and due to increase in TP the stomata open.

Factors affecting Transpiration-

---prepared by: Subodh Khanal
Transpiration is affected by following factors-

a) External or environmental factors, and

b) Internal or structural factors.

External or environmental factors-

1. **Humidity in air**: less humidity in the air increases the rate of transpiration. High humidity in the air decreases the rate of transpiration.

2. **Temperature**: the rate of transpiration is directly proportional to the temperature of atmosphere.

3. **Light**: transpiration increases in high light intensity and decreases in low intensity.

4. **Wind velocity**: High wind velocity increases the rate of transpiration and low wind velocity decreases the rate of transpiration.

5. **Water content in soil**: If excess water is present in the soil, plants will absorb more water and the rate of transpiration also increases. In water deficient soil the rate of transpiration decreases.

Internal factors-

1. Presence of cuticle on epidermis
2. Hairy leaves
3. Presence of sunken stomata
4. Number of stomata on leaves

<table>
<thead>
<tr>
<th>Transpiration</th>
<th>Guttation</th>
</tr>
</thead>
<tbody>
<tr>
<td>(i) It occurs through stomata, cuticle and lenticels.</td>
<td>(i) It occurs through hydathodes in the leaves.</td>
</tr>
<tr>
<td>(ii) Water is lost in the form of water vapour.</td>
<td>(ii) It is exuded in the form of liquid.</td>
</tr>
<tr>
<td>(iii) It occurs only in day time.</td>
<td>(iii) It takes place either in the morning or during the night.</td>
</tr>
<tr>
<td>(iv) The stomata of leaves usually remain open during day and get closed at night.</td>
<td>(iv) The hydathode remains open whole day and night.</td>
</tr>
<tr>
<td>(v) Water is pure and contains no salt.</td>
<td>(v) Oozed out water is not pure and contains inorganic and organic substances.</td>
</tr>
<tr>
<td>(vi) Major loss of water takes place through stomata.</td>
<td>(vi) It occurs through hydathodes.</td>
</tr>
<tr>
<td>(vii) It takes place in all higher terrestrial plants.</td>
<td>(vii) It takes place mostly in herbaceous plants.</td>
</tr>
<tr>
<td>(viii) Transpiration maintains the temperature of the plant,</td>
<td>(viii) It has no relation with the temperature.</td>
</tr>
<tr>
<td>(ix) Root pressure is not involved, e.g. all vascular plant</td>
<td>(ix) It takes place due to development of root pressure.</td>
</tr>
</tbody>
</table>

---prepared by: Subodh Khanal---
Why transpiration is called a necessary evil?

The loss of water in the form of vapors from aerial parts of the plant is called transpiration. Transpiration is said to be a necessary evil because it is an inevitable, but potentially harmful, consequence of the existence of wet cell surfaces from which evaporation occurs. Loss of water from the plant results in wilting, serious desiccation and often death of a plant if a condition of drought is experienced. There is strong evidence that even mild water stress results in reduced growth rate and in crops to economic losses through reduction of yield. The stomata are primarily meant for absorption of CO₂ but these also help in exchange of gases, but at the same time water vapors also escape through stomata. Thus transpiration is described as necessary evil because it is an inevitable process but potentially harmful. Loss of water can lead to wilting, serious desiccation, and often death of a plant, if there is shortage of water. There is good evidence that even mild water stress results in reduced growth rate, and reduction in yield. Despite its apparent inevitability, transpiration is also of great significance for the plant. Water is conducted in most tall plants due to transpiration pull.

Minerals dissolved in water are distributed throughout the plant body by transpiration stream. Evaporation of water from the exposed surface of cells of leaves has a cooling effect on plant. Wet surface of leaf cells allow gaseous exchange. Transpiration affects indirectly the processes of respiration and photosynthesis. Transpiration is supposed to help the plant in the following ways.

1. Excess water getting into the plant might decay the cells. Transpiration prevents it by removing the extra water.
2. Extra quantity of water, if retained would disturb the osmotic relationship between cells. This is prevented by transpiration.
3. Transpiration stream helps in the distribution of nutrients to all the parts of the plant body.
4. Fresh and cool water reaches all the parts of the plant body and this reduces the metabolic heat acting as a coolant.
5. Transpiration indirectly helps in the uptake of salts as the latter get into the plant together with water.
6. Upward movement of water (Ascent of sap) is due to the suction force created by transpiration.

Some of the recent researches however, have indicated that transpiration after all is not advantageous as was thought previously. The fact of water escape is a consequence of the anatomical construction of the leaf. The anatomical structure of the leaf is necessary for photosynthesis and respiration. When there is an escape route water also escapes. Transpiration does not remove excess of water. The plants in fact are forced to absorb more water because most of it will be lost by way of transpiration. That is why transpiration is often called a necessary evil.
5. Photosynthesis

5.1 concept significance, absorption spectra, photosynthetic pigments, light reaction, cyclic and non cyclic photophosphorylation

5.2. C3, C4 and CAM cycle

5.3 factors affecting photosynthesis

The word “photos” mean light and “synthesis means putting together. Because of the production of energy rich substances in the presence of light by chlorophyll, this process is called photosynthesis. Thus, the formation of carbohydrates from CO2 and water by illuminated green cells is called as photosynthesis.

In other words photosynthesis is a process in which carbon dioxide is converted into carbohydrates in the presence of water and chlorophyll by all organisms containing chlorophyll.

\[
6\text{CO}_2 + 12\text{H}_2\text{O} \rightarrow \text{C}_6\text{H}_{12}\text{O}_6 + 6\text{H}_2\text{O} + 6\text{O}_2
\]

Chlorophyll

As you know it is during this process 686 K.cal of energy is stored in chemical form.

SIGNIFICANCE OF PHOTOSYNTHESIS TO MANKIND

1. It maintains the equilibrium of O2 and CO2 in the atmosphere.
2. It provides food either directly as vegetable or indirectly as meat or milk of animals which in turn are fed on plants.
3. Life on earth is possible because of photosynthesis.
4. All useful plant products are derived from the process of photosynthesis e.g. timber, rubber, resins, oils, fiber, etc.
5. Photosynthesis decreases the concentration of CO2 which is being added to the atmosphere by the process of respiration of living beings and burning of organic fuels.

<table>
<thead>
<tr>
<th>Transpiration</th>
<th>Evaporation</th>
</tr>
</thead>
<tbody>
<tr>
<td>It is vital physiological process</td>
<td>It is purely physical process</td>
</tr>
<tr>
<td>Living tissues are essential</td>
<td>Any living or non living cells can perform</td>
</tr>
<tr>
<td>It is regulated by the activity of guard cells</td>
<td>There is no any specialized structure for evaporation</td>
</tr>
<tr>
<td>Different types of pressure(O.P., DPD) are essential</td>
<td>Such pressures are not essential</td>
</tr>
<tr>
<td>Helps to keep the surface of leaves smooth and protect it from burning</td>
<td>When it occurs the surface becomes completely dry</td>
</tr>
<tr>
<td>Water is lost in the form of water vapor</td>
<td>There is conversion of any liquid into vapor without necessarily reaching the boiling point</td>
</tr>
</tbody>
</table>
PHOTOSYNTHETIC APPARATUS:
In plants chloroplast are the organelles involved with photosynthesis process. Park and beggins (1964) called photosynthetic units present in granum discs quantasome.

STRUCTURE

Chloroplast
Ultra structure: Electron microscopic studies reveal that chloroplast is composed of following two parts.
1. Limiting membrane: chloroplast is bounded by double membraned lipoprotein a covering. About 40-60 Å thick.
2. Stroma or matrix: The stroma is the inner matrix of the chloroplast which fills the inner hollow space. It contains starch granules and osmophilic droplets.

Photosynthesis takes place in grana.
(i) Light reaction: Takes place in grana.
(ii) Dark reaction: Takes place in stroma.
2. During photosynthesis chloroplast absorb CO2 from atmosphere and photolyse H2O to release O2. This O2 is utilized by living beings during respiration. Thus, chloroplast controls the concentration of O2 and CO2 in the atmosphere.
3. During light reaction of photosynthesis, phosphorylation of ADP takes place, which results ATP generation. This ATP and NADPH2 are required for the reduction of CO2 during dark reaction of photosynthesis.

PHOTOSYNTHETIC PIGMENTS AND LIGHT HARVESTING COMPLEXES
Chloroplast or chromatophores contain pigments which convert light energy into chemical energy during photosynthesis. There are three types of pigments in photosynthetic cells: 1. Chlorophylls, 2.Carotenoids and 3.phycobilins.

1. Chlorophylls are found within specialized structures called chloroplast, while phycobilins are found within phycobilisomes. Chlorophylls and carotenoids are insoluble in water. Chlorophyll (G.K., chlor=green, phyll=leaf): Chlorophyll is a green pigment. Found within chloroplast of all green plants and in involved in photosynthesis. It is made up of 5 types of elements C, H, O, N, and Mg.
2. Carotenoids: It is yellow or orange colored pigment usually found in close association with chlorophylls. They occur in thylakoids and act as accessory pigment of photosynthesis. It absorbs light energy in the mid region of visible spectrum and transfer their absorbed energy to chlorophyll molecules. They pick up nascent O2 released during photo oxidation of water and change them into molecular state. Thus, they protect the chlorophyll molecules from photo-oxidation.

3. Phycobilins:- are red or blue coloured pigments bound in BGA. viz, phycocyanin, Phycoerythrin.

Absorption spectra:

<table>
<thead>
<tr>
<th>Color</th>
<th>Frequency</th>
<th>Wavelength</th>
</tr>
</thead>
<tbody>
<tr>
<td>violet</td>
<td>668–789 THz</td>
<td>380–450 nm</td>
</tr>
<tr>
<td>blue</td>
<td>606–668 THz</td>
<td>450–495 nm</td>
</tr>
<tr>
<td>green</td>
<td>526–606 THz</td>
<td>495–570 nm</td>
</tr>
<tr>
<td>yellow</td>
<td>508–526 THz</td>
<td>570–590 nm</td>
</tr>
<tr>
<td>orange</td>
<td>484–508 THz</td>
<td>590–620 nm</td>
</tr>
<tr>
<td>red</td>
<td>400–484 THz</td>
<td>620–750 nm</td>
</tr>
</tbody>
</table>

**Quantum:** Discrete energy particles present in the radiant energy including light is called as quantum. It is the unit of light energy dependent on the frequency of wavelength of radiation.

**Quantasome:** minimum amount of pigment molecules working together collectively to make the photochemical reaction effective where 250 molecules of chlorophyll, 8 molecules of oxidizing agents, 8 molecules of cytochrome a and 16 molecules of cytochrome b are present. All together the size is 18*16*10 nm and is arranged tightly in monolayer of thylakoids.

**Quantum requirement:** the number of photons or quanta required to release one molecule of oxygen in photosynthesis is called quanta requirement. 2quanta are required for transfer of each atom of H+ ion from H2O to CO2 during photosynthesis.

**Quantum yield:** the number of oxygen molecule released per photon of light in photosynthesis is called quantum yield.
MECHANISM OF PHOTOSYNTHESIS
Photosynthesis is a multistep oxidation-reduction reaction. According to modern scientists, the following three processes will take place during photosynthesis:
1. First of all plants absorb light energy with the help of their pigment systems.
2. Then absorbed light energy is converted into chemical energy.
3. Finally synthesis of carbohydrates takes place.
Of the above three processes, first two takes place in the presence of light hence it is called as light reaction, whereas third one is very complex process which does not require light hence called as dark reaction. Thus photosynthesis consists of two successive series of reactions:
1. Light or Hill reaction
2. Dark reaction or Blackmann’s reaction.
The light dependent phase can be described under the following sub headings:

Light reaction takes place in grana of chloroplast and it requires light hence it is called light reaction. In this reaction light energy is utilized and formation of ATP and reducing power (NADPH + H⁺) takes place. This NADPH + H⁺ is the reduced part of redox system NADP⁺/NADPH. The electrons required for the conversion of NADP⁺ into NADPH comes from water. Thus, in this process water functions as electron donor. Light reaction was discovered by Robert Hill (1937) hence it is also known to be as Hill reaction.

A. Red drop and Emerson’s enhancement effect
Robert Emerson (1932) and Lewis et al. (1943) while determining the quantum yield of photosynthesis in Chlorella, Plant pigment by utilizing monochromatic light of different wavelength from 400-760nm. They found that a drop in region of 440-520nm but the curve remained constant in the region of 600-680 nm but dropped sharply on the wavelength 680 nm. This sharp decline in the quantum yield beyond red region of spectrum was called red drop.

The further extended experiment concluded that if light of shorter wavelength was super imposed with the light of far red wavelength, the rate of photosynthesis was greater that could be expected from adding rates found when the light was given alone. This enhancement by light of shorter wavelength was called as Emerson’s Enhancement Effect.

He formulated as follows:

\[
E = \Delta O_2 \text{(in combined beam)} - \Delta O_2 \text{(Short wavelength beam alone)}
\]

\[
\Delta O_2 \text{(longer wavelength in beam alone)}
\]

Where \(\Delta O_2 = \text{rate of oxygen evolution}\)
B. Pigment system

Photosystems (ancient Greek: photos = light and systema = assembly) are functional and structural units of protein complexes involved in photosynthesis that together carry out the primary photochemistry of photosynthesis: the absorption of light and the transfer of energy and electrons. They are found in the thylakoid membranes of plants, algae and cyanobacteria (in plants and algae these are located in the chloroplasts), or in the cytoplasmic membrane of photosynthetic bacteria.

Photosystem I

- It is located in both grana and stroma and is active in both red and far wavelength light.
- It carries single cyclic phosphorylation.
- It comprises 100 chlorophyll a molecules, some beta carotene attached to proteins, 2 phylloquinones, Fe-S complex and cytochrome bf complex.
- This system uses light of wavelength 700 nm i.e. light harvest center is P700.

Photosystem II

- It is located in the inner surface of thylakoids and is inactive in far red light.
- It carries non cyclic phosphorylation.
- It comprises of 40-50 chl a molecules with little chl. B molecules, beta carotenes, pheophytins, maganoprotein, cl ion, cytochrome b559, cytochrome b6, 6-7 polypeptides
- It absorbs light 680 nm.

C. photolysis of water:

a. oxidized chlorophyll a (chl+) has a lack of electrons which are replaced as H2O is split:

\[ \text{H}_2\text{O} \rightarrow 2 \text{H}^+ + 2 \text{e}^- + \frac{1}{2} \text{O}_2 \]

. H+:

1) remain in thylakoid interior, lowering pH
2) contributing to chemiosmotic gradient
3) used in phosphorylation of ATP

---prepared by: Subodh Khanal
d. 2 electrons: replace electrons lost by chlorophyll a to ETS: chl a+ + 2 e-s ---> chl a

e. 1/2 O2: lost to environment as a waste product

\[
\begin{align*}
4H_2O & \rightarrow 4H^+ + 4OH^- \\
4OH^- + 2Z & \rightarrow 2Z(OH)^2+4e^- \\
2Z(OH)^2 & \rightarrow 2Z+2H_2O + O_2
\end{align*}
\]

\[
2H_2O \rightarrow 4H^+ +4e^-+O_2
\]

- *electron transport system*: proteins embedded in thylakoid membrane transfer energy along a pathway in a series of redox reactions:

  a. from PsII to PsI
  b. from PsI to NADP+
  c. some energy used to pump H+s from stroma to thylakoid interior lowering pH
  d. contributing to chemiosmotic gradient used in phosphorylation of ATP

D. phosphorylation( discussed below)

Steps of Light Reaction

1. **Absorption of light energy by chloroplast**: During photosynthesis first of all different kinds of chlorophyll molecules of leaves absorb light of different wavelengths of visible part (between 360nm to 810nm) of the spectrum and transfer it towards *reaction centre* of the pigment systems.

2. **Transfer of light energy from accessory pigment to chlorophyll-a**:

   All the photosynthetic pigments other than Chl-a are called as antenna or accessory pigments. These antenna chlorophyll absorb light energy and transfer them into *photoreaction centre* or energy trapping centre. In PS-I energy trapping centre is P700 whereas in PS-II it is P680.

3. **Activation of chlorophyll-a molecules by photons of light energy**:

   ![Diagram](Light.png)

   Chlorophyll – a (Ground State) \( \xrightarrow{\text{Light}} \) Chlorophyll – a (Excited State)

   Chlorophyll – a \( \xrightarrow{} \) (Chlorophyll – a)\(^+\) + e\(^-\)

4. **Photolysis or photochemical oxidation of water and evolution of oxygen**: The photolysis of water molecules takes place in pigment system II in presence of Mn\(^{++}\) and Cl\(^-\) ions. According to Von Niels and Frank (1941) excited molecules of chlorophyll-a react with water. In this state PS-II become activated and water molecules (H\(_2\)O) dissociated to form H\(^+\) and OH\(^-\) ions. This process is known *photochemical breakdown* or *photolysis of water*. OH\(^-\) ions releases electrons (e\(^-\)) and finally a molecule of water is formed and O\(_2\) gas is liberated. It is
believed that photolysis of water takes place due to presence of a strong oxidant which is not yet identified. And named as ‘Z’.

\[
\begin{align*}
4H_2O & \rightarrow 4H^+ + 4OH^- \\
4OH^- + 2Z & \rightarrow 2Z(OH)^2 + 4e^- \\
2Z(OH)^2 & \rightarrow 2Z+2H_2O + O_2 \\
2H_2O & \rightarrow 4H^+ + 4e^- + O_2
\end{align*}
\]

The released H+ ions are used for reduction of NADP or NAD

5. **Electron transport and the production of assimilatory power (NADPH + H+ and ATP)**: The electron expels from P 680 and P 700 after travelling through Electron Transport System (E.T.S) of photosynthesis, are either assumed in reducing NADP+ to NADPH + H+ or cycled back. The extra light energy is used in the formation of ATP molecule s at different place during its transport. It is called as photosynthetic phosphorylation.

**Photophosphorylation**

The process of formation of ATP from ADP and inorganic phosphate (Pi) is called photophosphorylation.

\[
\begin{align*}
ADP + Pi & \rightarrow ATP \text{ or } ADP + Pi \rightarrow ATP
\end{align*}
\]

Light, Mn, chl

According to **Arnon and associates**, photophosphorylation or E.T.C. Involves the following two processes:

i. Non-cyclic photophosphorylation,

ii. Cyclic Photophosphorylation.

In **cyclic photophosphorylation** the electrons lost by PS-I is cycled back to it, whereas in **non-cyclic photophosphorylation**, one electron is lost it doesn’t enter into PS-II, thus it involves both PS-I and PS-II.

(i) **Non-cyclic photophosphorylation**:

- **Hill** and **Bendal** (1960) and **Robinowitch** and **Govindjee** (1965) have proposed **Z-scheme** to explain the process of photophosphorylation.
- It is associated with both PS-I and PSII which is also called as Z scheme of electron transport
- Water is ultimate source of electrons i.e. 2H+ + 2e- are liberated by water molecule. Excited electrons are transported over to Z scheme where the oxygen is liberated.
- Flow of electron is unidirectional i.e. uphill and down hill migration of electrons
- The primary electron acceptor is Q. electrons are carried one after another though plasto quinine, cyt.b, cyt. f, PC, in PSII and X**ferredoxin reducing substance (FRS)** and iron containing protein called **ferredoxin**. Finally PC hands the electron to P700 in PSII. NADP is the final acceptor of electron in PSI. NADP is reduced to NADPH with the help of H+ released from H2O.
- The carriers are **cytochrome-b** (Cyt-b), **plastoquinone (PQ)**, **cytochrome-f**.(Cyt-f) and **plastocyanin (PC)**.
(ii) Cyclic Photophosphorylation:

- The cyclic photophosphorylation take place under certain condition e.g., when the amount of available NADP is low or PS-II is absent. It involves PS-I and therefore, photolysis of water and the consequent evolution of $O_2$ does not take place.
- Non-cyclic electron transfer does not take place and NADPH is not formed.
- The process doesn’t take part in photosynthesis except in certain bacteria.
- The process is not connected to photolysis of water, no oxygen is evolved.
- The electron lost by P 700 is cycled back to it through $X$, FRS, FD and cytochrome-$b_6$, cytochrome –f and plastocyanin. 2ATP molecules are synthesized from 2ADP and inorganic phosphate when electron is transferred from cytochrome–$b_6$ to PQ and from cytochrome-$b$ to cytochrome-$f$. 

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Thus, from the above description it is clear that photochemical reaction takes place during light reaction results:

(i) Photolysis of water and release of $\text{O}_2$

(ii) Formation of 3 ATP

(iii) Formation of 2NADPH$_2$

ATP and NADPH are used in the reduction of $\text{CO}_2$ during dark reaction. Similarly ATP and NADPH$_2$ function as carrier of energy of sunlight and transfer it up to dark reaction. ATP together with NADPH$_2$, called as **assimilatory power** and NADPH$_2$ is called as **reducing power**.

**DARK REACTION**

It is also known as **Blackmann’s reaction** or **thermochemical reaction**. In this phase, the NADPH + H$^+$ and ATP produced during light phase are used in the reduction or fixation of $\text{CO}_2$ into carbohydrates. This reaction takes place in **stroma** of chloroplast.
CALVIN CYCLE OR C₃-CYCLE

This method of CO₂ fixation is described by Calvin, Benson and Bassham (1957). As first stable product of this reaction is phosphoglycericacid (PGA), which is a three carbon compound, this cycle is known to be as C₃-cycle and plants with C3 cycle as C₃ plants.
(i) 6RuDP + 6CO\textsubscript{2} \xrightarrow{\textit{Carboxydismutase}} 12PGA (RuDP-C)

(ii) 12PGA + 12ATP \xrightarrow{\textit{Kinase}} 12 1,3-DPGA + 12ADP

(iii) 12 1,3-DGPA + 12NADPH\textsubscript{2} \rightarrow 12 3-PGAld + 12NADP + 12H\textsubscript{3}PO\textsubscript{4}

(iv) 5PGAld \xrightarrow{\textit{Isomerase}} 5DHAP

(v) 3PGA + 3DHAP \xrightarrow{\textit{Aldolase}} 3F-1,6-DP \xrightarrow{\textit{Phosphate}} 3F-6-P+3Pi

(vi) 1F-6-P \xrightarrow{\textit{Transketolase}} 1Hexose

(vii) 2F-6-P + 2,3 –PGAld \xrightarrow{\textit{Transketolase}} 2E-4-P + 2Xy-5-P

(viii) 2E-4 –P + 2DHAP \xrightarrow{\textit{Aldolase}} 2S-1,7-DP + 2H\textsubscript{2}O \xrightarrow{\textit{Phosphatase}} 2S-7-P + 2H\textsubscript{3}P\textsubscript{04}

(ix) 2S-7-P + 2PGAld \xrightarrow{\textit{Transketolase}} 2Ribose-5-P+ 2Xy-5-P

(x) 2 Ribose-5-P \xrightarrow{\textit{Isomerase}} 2Ribusole-5-P

(xi) 4 Xy-5-P \xrightarrow{\textit{Epimerase}} 4 Ribulose – 5 – P

(xii) 2Ribose \xrightarrow{\textit{Epimerase}} 2 Ribose – 5 – P

(xiii) 6 Ribusole – 5 – P + 6 ATP \xrightarrow{\textit{Phosphatase}} 6 Ribusole-1,6-DP

---prepared by: Subodh Khanal---
PHOTORESPIRATION It has been observed that light affects respiration and the rate of respiration in light may be three to five times higher than the respiration in darkness. Such type of respiration is called photorespiration. In photorespiration, temperature plays a very vital role, its rate being very high in between 25-35°C. It also depends upon the concentration of oxygen and increases with increasing oxygen concentration. Even up to 100%. In normal respiration the respiratory substrate is sucrose which in photorespiration glycolic acid (2 carbon compound)

---prepared by: Subodh Khanal
serve as a substrate.

Main features of P.R. are
1. It takes place in the presence of light.
2. glycolate serves as substrate for photorespiration.
3. Photorespiration takes place in peroxisomes. Chloroplast and mitochondria are also involved in this process.
4. It occurs in some plants like Beet, Rice, Bean, etc.
5. Photorespiration increases with the availability of O2
6. It is pronounced in C3 plants and negligible in C4 plants.
7. Toxic H2O2 is formed during oxidation of the substrate
8. End-products are CO2.
9. It is wasteful method and does not produce energy.

**HATCH AND SLACK CYCLE OR C_{4}– CYCLE**

Initially it was believed that CO₂ fixation takes place only by Calvin cycle. But in 1954, in addition to Calvin cycle, an alternate pathway to CO₂ fixation in photosynthesis was discovered by Kortschak et al. who reported the formation of C₄ dicarboxylic acid as primary product of photosynthesis in sugarcane. M.D Hatch and C.R.Slack (1966) proposed an alternative pathway of CO₂ fixation which is now known as Hatch and Slack pathway or C₄-dicarboxylic acid pathway or C₄-cycle or β-carboxylation cycle.

*This cycle is known as C₄ cycle because first stable product of this cycle is a four carbon compound known as oxaloacetic acid (OAA).

**Mechanism of C₄-cycle:**

There are two carboxylation reaction takes place in C₄-cycle. **First carboxylation reaction** takes place in mesophyll chloroplast and **second carboxylation** takes place in bundle sheath cells in the following step wise reaction:
**Occurrence**: \( C_4 \)-cycle is found in the members of the family gramineae e.g. sugarcane, maize, etc. It is also found in the members of the family Cyperaceae, Azoaceae, Amaranthaceae, Chenopodiaceae, Euphorbiaceae, and Nyctaginaceae.

There are several variants of this pathway:

1. The 4-carbon acid transported from mesophyll cells may be malate as above, or may be aspartate.
2. The 3-carbon acid transported back from bundle-sheath cells may be pyruvate as above, or alanine.
3. The enzyme which catalyses decarboxylation in bundle-sheath cells differs. In maize and sugarcane, the enzyme is NADP-malic enzyme, in millet, it is NAD-malic enzyme, and in *Panicum maximum* it is PEP carboxykinase.

**Characteristic Features of C₄ plants:**

1. C₄ plants are usually found in tropical region where temperature is between 30-35ºC and light intensity is very high.
2. Photorespiration does not take place in C₄ plants or the rate of photorespiration is very low.
3. C₄ plants have high photosynthetic rate (40-80 mg CO₂ per hour) whereas the rate of photosynthesis in C₃ plants is 10-15 mg CO₂ per hour.
4. The leaves of C₄ plants exhibit specific histological structure. The *vascular bundle (V.B.) of leaves of C₄ plants* is bounded by *bundle sheath cells*. The cells of bundle sheath are bounded by mesophyll cells. Bundle sheath cells have different types of chloroplast. This type of anatomical structure is known as *Kranz anatomy*.
5. Leaves of C₄ plants contains two types (dimorphic) of chloroplast:
   (i) *Mesophyll Chloroplast*: It is smaller, grana is present there and starch grains are absent
   (ii) *Bundle sheath Chloroplast*: It is larger in size, lacking grana and possessing starch grains. *Malic acid* and *aspartic acid* is formed from *pyruvic acid* within mesophyll cells.
6. (i) *Ribulose diphosphate carboxylase or Rubisco*: This enzyme is found within the *chloroplast of bundle sheath* cells. It catalyses the *oxidative decarboxylation of malic acid* to produce *pyruvic acid* and reduces CO₂ to C₃ cycle.
   (ii) *Phosphoenol pyruvic carboxylase* (PEP-C): This enzyme is found in the chloroplast of *mesophyll cells* and it reduces atmospheric CO₂ by C₄ cycle.
7. There are two pathways of CO₂ fixation in C₄ plants.
   (i) C₄-cycle (takes place in mesophyll cells) and
   (ii) C₃-cycle (takes place in bundle sheath cells)

**Biological Significance of C₄-cycle:**

1. Production in C₄ plants is 2-3 times greater than C₃ plants.
2. C₄ plants can photosynthesize even in the presence of very low concentration of CO₂. C₄ plants possessing a very efficient enzyme system to utilize least amount of CO₂. This enzyme system is known as phosphoenol pyruvic carboxylase (PEP-C).

3. PEP-C enzyme have high affinity with CO₂ than RuDP-C enzyme, hence plants can even fix CO₂ during short day conditions when very least concentration of CO₂ is available.

4. The rate of photorespiration in C4 plants is very low (negligible) hence the rate of photosynthesis will be higher in these plants.

CRASSULACEAN ACID METABOLISM OR CAM CYCLE

It occurs mostly in succulent plants which grow under semi-arid conditions. This mode of CO₂ fixation takes place during night (dark) because the stomata of leaves of these plants remain open only during night. These plants absorb CO₂ during night and convert it into malic acid which is then stored in vacuoles. During day time (light) decarboxylation of malic acid takes place and CO₂ is released. This CO₂ is utilized by C₃-cycle. Since the cycle was first observed in the plants belonging to family Crassulaceae e.g. Bryophyllum, Sedum and Kalanchoe, etc. It was named as Crassulacean Acid Metabolism (CAM). Similar metabolism has been reported in the plants belonging to following families:

1. **Dicot Families**: Crassulaceae e.g. (sedum, Opuntia) Aizoaceae, Asclepiadaceae, Caryophyllaceae, Chenopodium, compositae, convolvulaceae, Euphoebiaceae, Vitaceae, etc.
2. **Monocot Families**: Liliaceae, Orchidaceae.
3. **Pteridophytes**: Polypodiaceae.

**Characteristic Features of CAM plants**

1. The stomata remain closed during day (light) and open at night (dark).
2. CO₂ fixation takes place in chlorophyll containing cells of leaves and stem during night (dark) and malic acid synthesis takes place.
3. Malic acid formed during dark (night) is stored in large vacuoles.
4. During day time decarboxylation of malic acid takes place and CO₂ gas is released. This CO₂ is converted into sucrose and storage glucans (e.g. Starch) by C₃-cycle.

Thus, CAM plants show diurnal cycle of organic acid formation i.e. they fix atmospheric CO₂ during night by CAM and fix internally borne CO₂ by C₃-cycle during day time.

**Mechanism of CAM cycle**

The cycle is completed in following two parts:

1. Acidification and 2. Deacidification

1. **Acidification**: Acidification takes place during following steps:
(i) The stored carbohydrates are converted into **phosphoenol pyruvic acid** (PEP) through glycolysis. As stomata opens during night, the CO₂ diffuses freely into the leaf through open stomata at night.

(ii) The CO₂ combine with PEP in the presence of **phosphoenol-carboxylase** (PEP-C) enzyme to produce **oxaloacetic acid** (OAA).

(iii) The **oxaloacetic acid** (OAA) is not reduced into **MALIC ACID** in the presence of **malic dehydrogenase enzymes**. This reaction is facilitated in presence of reduced NADP⁺ (=NADPH + H⁺) formed during glycolysis. This malic acid, thus produced in dark as a result of acidification is stored in the vacuoles. The oxaloacetic acid (OAA) may also be interconverted into **aspartic acid**

2. **Deacidification**: The decarboxylation of **malic acid** into **pyruvic acid** and CO₂ in presence of light is called **deacidification**.

CO₂ liberated is fixed by C3 cycle on coming next night this starch is converted into PEP, and is thus ready to accept atmospheric CO₂.
Blackman’s law of limiting factors

Blackman (1905) proposed the law of limiting factors according to which when process is conditioned to its rapidity by a number of factors, the rate of process in limited by the pace of the slowest factor. Blackman’s law of limiting factor is modification of Leibig’s law of minimum, which states that rate of process controlled by several factors, is only as rapid as the slowest factor permits. Theory of three cardinal points was given by Sachs in 1860. According to this concept, there is minimum, optimum and maximum for each factor For every factor, there is a minimum value when no photosynthesis occurs, an optimum value showing highest rate and a maximum value, above which photosynthesis fails to take place.

External factors

1. Light

In photosynthesis light is converted to chemical energy in the food formed. It can be studied under three headings (i) Light intensity (ii) Light quality and (iii) Light duration (i)

(i) Light intensity.

(ii) Light quality. Blue and red light of the spectrum is said to be the best light for the photosynthesis. The green light has inhibitory effect. On the other hand, plants growing in deep water absorb green light.

(iii) Light duration. Plants getting average light of 10-12 hours a day show higher rate of photosynthesis. Apple trees were found to carry on photosynthesis in continuous light for eighteen days (Bohning 1949).

2. Carbon dioxide

Carbon dioxide is present in low concentration and forms about 0.032% of the total atmosphere. Increased concentration of CO2 with other factors not becoming limiting rate of the process enhances. However, very high concentration of CO2 becomes toxic to the plants. It is doubtful whether CO2 is a limiting factor under field conditions.

3. Water

Water deficiency may decrease the rate as it is one of the raw materials for the process. Less availability of water may further check the rate by closing the stomata thereby affecting the entry of CO2.
4. Temperature

5. Oxygen

Excess of O2 may become inhibitory for the process. An increase in oxygen concentration decreases photosynthesis and the phenomenon is called Warburg effect. The explanation of this problem lies in the phenomenon of photorespiration.

6. CO₂ Concentration

- CO₂ is essential for the reduction of ribulose bisphosphate during the Calvin cycle for the production of carbohydrates.
- As long as there are no other limiting factors an increase in CO₂ concentration up to about 0.05% will increase photosynthetic rate.
- At this level photosynthetic rate reaches plateau.
- Concentrations above 0.1% can damage leaves.
- Optimum concentration of CO₂ is therefore just below 0.1%
- In dense, warm, well lit vegetation areas low levels of CO₂ limit photosynthesis. Why?

7. Osmotic relations

Availability of water is affected indirectly with respect to osmotic relations of the plants. Internal factors

1. Protoplastic factors: There is some unknown protoplasmic factor which affects the rate of photosynthesis. It takes some time to initiate the process in seedlings even if the chlorophyll has appeared. Same is true, if the plant is shifted to light from prolonged darkness.

2. Chlorophyll contents: Quantity of chlorophyll seems to affect the process. In variegated leaves and green leaves, assimilation per unit leaf area has been found to be the same provided other factors are not limiting. The amount of CO₂ fixed by a gram chlorophyll in an hour is called as photosynthetic number or assimilation number.

3. Accumulation of products: Accumulation of photosynthetic products, if not consumed or translocated results in stoppage of process gradually.

4. Structure of leaves: Characters like structure, position and distribution of stomata, intercellular spaces, vascular tissues have been noticed to affect the process directly.

What is the Significance of Photosynthesis?

i. Helps in conversion of solar energy into organic matter.
ii. Consumes atmospheric carbon dioxide and yields carbohydrates and molecular oxygen.
iii. Evolves molecular oxygen for use by other living organisms and maintains the level of atmospheric oxygen which is continuously consumed by plants and animals during respiration.
Photorespiration is a wasteful process because G3P is created at a reduced rate and higher metabolic cost (2ATP and one NADPH) compared with RuBP carboxylase activity. G3P produced in the chloroplast is used to create "nearly all" of the food and structures in the plant. While Photorespiratory carbon cycling results in G3P eventually, it also produces waste ammonia that must be detoxified at a substantial cost to the cell in ATP and reducing equivalents. Due to RuBisCO oxygenase activity is disadvantageous to plants for several reasons:

- Oxygenase produces a 2-carbon molecule instead of the 3-carbon molecule used by the Calvin Cycle to produce sugar and recycle RuBP; carbon is lost from the cycle
- The 2-carbon molecule is quickly metabolized into a molecule that is toxic to the plant at high concentrations; photosynthesis is inhibited.
- Salvaging this molecule is energetically expensive and results in only a 75% return of the 3-carbon sugar used in the Calvin Cycle.

### SOME PHOTOSYNTHETIC CHARACTERISTICS OF C-3 AND C-4 PLANTS

<table>
<thead>
<tr>
<th>Sn</th>
<th>CHARACTERISTIC</th>
<th>C-3</th>
<th>C-4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Leaf anatomy</td>
<td>No distinct bundle sheath of photosynthetic cells</td>
<td>Well organized bundle sheath, rich in organelles (starch storing chloroplasts)</td>
</tr>
<tr>
<td>2</td>
<td>Carboxylating enzyme</td>
<td>Ribulose diphosphate (RuDP) carboxylase</td>
<td>Phosphoenol pyruvate (PEP) carboxylase ± RuDP carboxylase</td>
</tr>
<tr>
<td>3</td>
<td>Theoretical energy requirement (CO2:ATP:NADPH)</td>
<td>1:3:2</td>
<td>1:5:2</td>
</tr>
<tr>
<td>4</td>
<td>Transpiration ratio ( g / H2O / g ) dry wt increase</td>
<td>450-950</td>
<td>250-350</td>
</tr>
<tr>
<td>5</td>
<td>Leaf chlorophyll a to b ratio</td>
<td>2.8 ±0.4</td>
<td>3.9±0.6</td>
</tr>
<tr>
<td>6</td>
<td>Requirement for Na as a micronutrient</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>7</td>
<td>CO2 compensation point (ppm CO2) i.e CO2 conc. at which net Photosynthesis ceases</td>
<td>30-37</td>
<td>0-10</td>
</tr>
<tr>
<td>8</td>
<td>Photosynthesis inhibited by 21% O2</td>
<td>Yes</td>
<td>No. &lt;3% largely independent of O2 concentration</td>
</tr>
<tr>
<td>9</td>
<td>Photorespiration detectable?</td>
<td>Yes</td>
<td>Only in the bundle sheath</td>
</tr>
<tr>
<td>10</td>
<td>Optimum temperature for photosynthesis</td>
<td>15-25°C</td>
<td>30-40°C</td>
</tr>
<tr>
<td>11</td>
<td>Dry matter production ton/ha/year</td>
<td>22±0.3</td>
<td>39+17</td>
</tr>
</tbody>
</table>

---prepared by: Subodh Khanal
## Others are

<table>
<thead>
<tr>
<th></th>
<th>C3 pathways</th>
<th>C4 pathways</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>The primary acceptor of CO2 is RUBP - a six-carbon compound.</td>
<td>The primary acceptor of CO2 is phosphoenolpyruvate - a three-carbon compound.</td>
</tr>
<tr>
<td>2.</td>
<td>The first stable product is 3-phosphoglycerate.</td>
<td>The first stable product is oxaloacetic acid.</td>
</tr>
<tr>
<td>3</td>
<td>It occurs only in the mesophyll cells of the leaves.</td>
<td>It occurs in the mesophyll and bundle-sheath cells of the leaves.</td>
</tr>
<tr>
<td>4.</td>
<td>It is a slower process of carbon fixation and photo-respiratory losses are high.</td>
<td>It is a faster process of carbon fixation and photo-respiratory losses are low.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>C-3</th>
<th>C-4</th>
<th>CAM</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Initial CO2 fixing enzyme</strong></td>
<td>ribulose 1,5 bisphosphate carboxylase/oxygenase</td>
<td>phosphoenolpyruvate carboxylase</td>
<td>phosphoenolpyruvate carboxylase</td>
</tr>
<tr>
<td><strong>Form of C in intermediates</strong></td>
<td>2 PGA (phosphoglycerate) molecules (3C)</td>
<td>4C molecules</td>
<td>4C molecules</td>
</tr>
<tr>
<td><strong>C stored in</strong></td>
<td>Starch after C-3</td>
<td>4-C molecules before C-3 and Starch after C-3</td>
<td>4-C molecules in vacuoles before C-3 and Starch after C-3</td>
</tr>
<tr>
<td><strong>Plant type</strong></td>
<td>all plants</td>
<td>grasses in warm climates</td>
<td>plants in hot, arid regions – succulents, cacti, agaves, some orchids, and bromeliads</td>
</tr>
<tr>
<td><strong>Location of process</strong></td>
<td>Chloroplasts of all phototrophs</td>
<td>mesophyll of Kranz-Crown/Halo plants</td>
<td>Chloroplasts nocturnal</td>
</tr>
<tr>
<td><strong>Evolution</strong></td>
<td>earliest, conserved</td>
<td>later, convergent</td>
<td>later, convergent</td>
</tr>
<tr>
<td><strong>Separation from C-3</strong></td>
<td>none</td>
<td>spatial</td>
<td>Temporal</td>
</tr>
<tr>
<td><strong>CO2 levels</strong></td>
<td>high in bundle sheath cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Advantages</strong></td>
<td>more efficient in cool, moist environments with moderate light intensity</td>
<td>more efficient in warm, dry environments with high light intensity</td>
<td>more efficient in hot, dry environments with high light intensity</td>
</tr>
</tbody>
</table>

---prepared by: Subodh Khanal
6. Respiration
6.1. Concept types and significance of respiration and respiratory quotient.
6.2. Mechanism of respiration: glycolysis and oxidation of pyruvic acid, Kreb’s cycle and its importance, ETS, oxidative photophosphorylation.
6.3. Factors affecting the rate of respiration

**Definition:** “Respiration is a process by which organic food materials such as sugar, fats, etc get successively oxidized to produce CO2, H2O and energy.”

\[
C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + 673Kcal\text{ energy}
\]

The overall reaction of cellular respiration is given as

\[
C_6H_{12}O_6 + 6O_2 + 38Adp +38iP \rightarrow 6CO_2 + 6H_2O + 38ATP
\]

The sites of respiration are cytoplasms and mitochondria. The organic compounds are broken down inside the cells by oxidation process, known as cellular respiration. The energy released is stored in pyrophosphate bonds of ATP.

\[
ADP + H_3PO_4 \rightleftharpoons ATP(ADP^\prime P)
\]

Energy stored in ATP is utilized for carrying out different cellular and biological activities because of this, energy is called energy currency of the cell.

The main features of respiration in plants are:

- Oxidation of organic compounds occurs in under aerobic conditions
- Complete oxidation occurs
- End products are CO2 & H2O
- Higher amount of (673 Kcal) energy is liberated out
- Process occurs in cytoplasm and mitochondria
- Chlorophyll pigment is not essential
- Various respiratory substance are: glucose, fructose, fats, protein, etc.
- The ratio of volume of CO2 released to the volume of O2 absorbed during respiration is called respiratory ratio or R.Q.

\[
R.Q. = \frac{\text{Volume of CO2 released}}{\text{Volume of O2 absorbed}}
\]

The significance of respiration is in energy supply (ATP), balance of O2 and CO2 in the environment and it provides several organic molecules required in the system.

---prepared by: Subodh Khanal
Significance of RQ

- By determining the value of RQ the nature of respiratory substrate can be known
  - Fats and protein RQ<1 as they require more oxygen.
  - For organic acids RQ>1 as low oxygen is required for oxidation.
  - In partial oxidation of carbohydrates are oxidized to organic acids in dark without CO₂ so RQ=0
  - During anaerobic respiration absence of O₂ makes RQ infinite.

MECHANISM OF RESPIRATION

Cellular respiration is a complicated process which is completed in many steps. For every step, a particular enzyme is required which works in a sequential manner one after the other. It is completed in 3 steps:

a) Glycolysis / EMP pathway
b) Oxidation of pyruvic acid
c) ETC & oxidative phosphorylation

GLYCOLYSIS/ EMP PATHWAY

In Greek language the word glucose means sugar and lysis means dissolution. If I say that glycolysis is a fermentive pathway would you agree? Reasons to support my statement are:

a) It does not involves O₂ intake
b) ATP generated is through substrate level phosphorylation.
c) Organic compound donates electrons and organic compound accepts it.

This process was discovered by three German scientists Embden, meyerhof and Parnas. On their name the pathway is also called EMP pathway.

All the reactions of glycolysis take place in the cytoplasm and through the glycolysis glucose is oxidized into pyruvic acid in presence of many enzymes present in the cytoplasm. Thus the process of sequential oxidation of glucose into pyruvic acid is known as glycolysis.
Energy production during glycolysis:

During glycolysis process two molecules of ATP are utilized to convert glucose into glucose-6-PO₄ & fructose-1,6 diphosphate where as 4 molecules of ATP and 2 molecules of NADH₂ are produced during following steps.

---prepared by: Subodh Khanal
(One molecule of NADH₂ gives three molecules of ATP by ETC)

<table>
<thead>
<tr>
<th>Reaction number</th>
<th>No. of ATP molecule produced</th>
</tr>
</thead>
<tbody>
<tr>
<td>(vii) 1,3 –DPG-Ald → 1,3-DPGA</td>
<td>2NADH₂(2*3) = 6ATP</td>
</tr>
<tr>
<td>(viii) 1,3 – DPGA → 3-PGA</td>
<td>2ATP = 2ATP</td>
</tr>
<tr>
<td>(xi) PEPA → Pyruvic Acid</td>
<td>2ATP = 2 ATP</td>
</tr>
</tbody>
</table>

As 2 molecules of ATP are utilized during glycolysis, thus net gain of ATP molecules during this process is 8 molecules of ATP
10 ATP – 2 ATP
Net gain of ATP = 8 ATP

**SIGNIFICANCE OF GLYCOLYSIS:**

a) Generate ATP
b) Precursor metabolic generation
c) Generates reducing power
d) Occurs in cytoplasm or cytosol in both aerobic and anaerobic condition.

**T.C.A. CYCLE/KREB’S CYCLE:**

This cycle was described for the first time by H.A.Kreb’s in 1943. It is also known as T.C.A. cycle because it produces tricarboxylic acids the process completes in **mitochondrial crests.**

All the chemical reaction of Kreb’s cycle can be summarized in following steps:

1. Aerobic oxidation of P.A
2. Condensation of Acetyl-CoA with oxalo-acetic acid
3. Isomerisation of citric acid into isocitric acid ,{(a) dehydration and (b) hydration)}
4. Oxidative decarboxylation of isocitric acid (a) dehydration and (b) decarboxylation)
5. Oxidative decarboxylation of α-Keto glutaric acid.
6. Conversion of succinyl CoA into succinic acid.
7. Dehydrogenation of succinic acid into fumaric acid
8. Hydration of fumaric acid into malic acid
9. Dehydrogenation of malic acid in OAA.

**Overall reaction of respiration is:**

Glycolysis + Kreb’s cycle = Glucose + 4ADP + 4H₃PO₄ + 8NAD⁺ + NADP⁺ +2FAD

→ 6CO₂ + 4 ATP + 8NADH + 10H⁺ +2NADPH + 2FADH₂
Because two molecules of P.A. which are formed by one molecule of glucose in glycolysis, enter into Kreb’s cycle for oxidation, a total of \(6\text{CO}_2\) molecule will be evolved.

\[2\text{PA} \times 3\text{CO}_2 = 6\text{CO}_2\]

All the NADH\(_2\) and FADH\(_2\) are oxidized to NAD and FAD through a chain of reaction c/a etc.in this process ATP molecules are released (1NADH\(_2\) = 3ATP, 1FADH\(_2\) = 2ATP). In the process of Kreb’s cycle 8 molecules of NADH\(_2\) =24ATP , 2FADH\(_2\) = 4 ATP and two molecules of ATP are synthesized from 2GTP.

---prepared by: Subodh Khanal
Significance of Kreb’s cycle:

- Synthesis of fatty acids from citrate.
- Synthesis of purine and pyrimidine nucleotide from alpha keto glutaric acid and oxalo acetic acid.
- OAA can be converted into glucose by glucogenesis.
- Succinyl co enzyme is a central intermediate in the synthesis of porphyrin ring of haeme group and also the precursor to form chlorophyll pigment.
- Gibberlin bio synthesis starts from acetyl co enzyme.
- Various amino acids synthesis occurs during the formation of organic acids in Kreb cycle.

ELECTRON TRANSPORT SYSTEM AND OXIDATIVE PHOSPHORYLATION

Electron Transport System (ETC)

During respiration simple carbohydrates and intermediate compounds like phosphoglyceraldehyde, pyruvic acid, isocitric acid, α ketoglutaric acid, succinic acid and malic acid are oxidized. Each oxidative step involves release of a pair of hydrogen atoms which dissociates into two protons and two electrons.

\[
2H \rightarrow 2H^+ + 2e^-
\]

These protons and electrons are accepted by various hydrogen acceptors like NAD,NADP, FAD etc. After accepting hydrogen atoms these acceptors get reduced to produce NADH₂, NADPH₂ and FADH₂. The pairs of hydrogen atoms released a series of coenzymes and cytochromes which form electron transport system, before reacting with O₂ to form H₂O.

\[
\frac{1}{2}O + 2H^+ + 2e^- \rightarrow H_2O
\]

\[
2NADH + O_2 + 2H^+ \rightarrow 2NAD^+ + 2H_2O
\]

The composition of each of the respiratory chain complexes is shown below and in Table 22-1 p. 806.

<table>
<thead>
<tr>
<th>Complex</th>
<th>Name</th>
<th>No. of Proteins</th>
<th>Prosthetic Groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complex I</td>
<td>NADH Dehydrogenase</td>
<td>46</td>
<td>FMN, 9 Fe-S centers</td>
</tr>
<tr>
<td>Complex II</td>
<td>Succinate-CoQ Reductase</td>
<td>5</td>
<td>FAD, cyt b₅₆₀, 3 Fe-S centers</td>
</tr>
<tr>
<td>Complex III</td>
<td>CoQ-cyt c Reductase</td>
<td>11</td>
<td>cyt b₇, cyt b₅₇, cyt c₁, Fe-S_Rieske</td>
</tr>
</tbody>
</table>

---prepared by: Subodh Khanal
Complex IV  |  Cytochrome Oxidase  |  13  |  cyt a, cyt a₃, Cu₄, Cu₅

Components of electron transport system: the electron transport system is made up of following enzymes and proteins:

1. Nicotinamide adenine dinucleotide (NAD).
2. Flavoproteins (FAD and FMN).
3. Fe-S protein complex.
4. Co-enzyme Q or ubiquinone.
5. Cytochrome-b
6. Cytochrome-c₁
7. Cytochrome-c
8. Cytochrome-a
9. Cytochrome-a₃

All the above enzymes are found in F₁ particles of mitochondria.

**Mechanism of action of electron transport system:**

During respiration electron pairs liberated from respiratory compounds are accepted by coenzymes like NAD or NADP and FMN etc. The transfer of electrons in all compounds except succinic acid takes place first in NAD⁺ or NADP⁺ and later on in FAD. The transfer of electrons from succinic acid takes place directly to the FAD and not through NAD⁺ or NADP⁺. Due to this reason only two molecules of ATP are formed in the formation of fumaric acid from succinic acid whereas in case of other compounds 3 ATP molecules are produced because these cases the electrons are first picked up by NAD.

Oxygen is thus the terminal electron acceptor of the mitochondrial respiratory chain.

**Oxidative Phosphorylation**

In all living beings ATP generated during oxidative breakdown of complex food products. This process of synthesis of ATP molecules from ADP and inorganic phosphate by electron transport system of aerobic respiration called as oxidative phosphorylation.

\[ \text{ADP} + \text{iP} \rightarrow \text{O}_2 \rightarrow \text{ATP} \]

E.T. Chain

The process of oxidative phosphorylation takes place in mitochondrial crests through electron transport chain.

During oxidative phosphorylation ATP molecules are produced during following steps:

I. When NADH₂ is oxidized to NAD by reacting with FAD.
II. When the electron transfer from cytochrome-b to cytochrome-c₁.
III. When the electron transfer from cytochrome-a to cytochrome-a₃.
Now it is clear that oxidation of one molecule of reduced NADH$_2$ or NADPH$_2$ results in the formation of 3 molecules of ATP while oxidation of FADH$_2$ leads to the formation of 2 molecules of ATP.

The table below describes the reactions involved when one glucose molecule is fully oxidized into carbon dioxide. It is assumed that all the reduced coenzymes are oxidized by the electron transport chain and used for oxidative phosphorylation.

<table>
<thead>
<tr>
<th>Step</th>
<th>coenzyme yield</th>
<th>ATP yield</th>
<th>Source of ATP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycolysis preparatory phase</td>
<td></td>
<td>-2</td>
<td>Phosphorylation of glucose and fructose 6-phosphate uses two ATP from the cytoplasm.</td>
</tr>
<tr>
<td>Glycolysis pay-off phase</td>
<td>2 NADH</td>
<td>4–6</td>
<td>Oxidative phosphorylation – Each NADH produces net 3 ATP due to NADH transport over the mitochondrial membrane</td>
</tr>
<tr>
<td>Oxidative decarboxylation of pyruvate</td>
<td>2 NADH</td>
<td>6</td>
<td>Oxidative phosphorylation</td>
</tr>
<tr>
<td>Krebs cycle</td>
<td></td>
<td>2</td>
<td>Substrate-level phosphorylation</td>
</tr>
<tr>
<td></td>
<td>6 NADH</td>
<td>18</td>
<td>Oxidative phosphorylation</td>
</tr>
<tr>
<td></td>
<td>2 FADH$_2$</td>
<td>4</td>
<td>Oxidative phosphorylation</td>
</tr>
<tr>
<td><strong>Total yield</strong></td>
<td></td>
<td><strong>36–38 ATP</strong></td>
<td>From the complete oxidation of one glucose molecule to carbon dioxide and oxidation of all the reduced coenzymes.</td>
</tr>
</tbody>
</table>
Factors affecting respiration

Internal factors:
- Protoplasmic factors
- Concentration of respiratory substrate.

External factors:
1. Temperature
2. Oxygen
3. Carbon dioxide
4. Water
5. Light
6. Injury
7. Inorganic salts
8. Mechanical effects
9. Respiratory inhibitors (cyanides, azides, CO)

Difference between aerobic and anaerobic respiration

<table>
<thead>
<tr>
<th>Aerobic Respiration</th>
<th>Anaerobic Respiration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Aerobic respiration takes place in the presence of free oxygen</td>
<td>1. Anaerobic respiration takes place in the absence of free oxygen.</td>
</tr>
<tr>
<td>2. The first step of this process (glycolysis) takes place in cytoplasm while the</td>
<td>2. The complete process is carried out outside the mitochondria i.e., in the cytoplasm.</td>
</tr>
<tr>
<td>second step (Krebs cycle) is carried out in mitochondria.</td>
<td></td>
</tr>
<tr>
<td>3. Glucose is completely oxidized into carbon</td>
<td>3. Glucose is incompletely oxidized into</td>
</tr>
</tbody>
</table>
dioxide and water.

4. 38 molecules of ATP are produced by the complete oxidation of one gram-mole of glucose.

carbon dioxide and ethyl alcohol.

4. Only 2 molecules of ATP are formed in this process.

<table>
<thead>
<tr>
<th>PHOTOPHOSPHORYLATION</th>
<th>OXIDATIVE PHOSPHORYLATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Photo means light and phosphorylation means addition of phosphate bond and in this particular case, addition of phosphate to adenosine diphosphate to make it adenosine triphosphate. This would require energy. Photons are the source of energy. This energy is harnessed by the chloroplasts. Part of this energy is used for splitting water into electrons and hydrogens (H+), and oxygen. And part of the light energy is used to make ATP form ADP and phosphate. Thus packaging of light energy into ATP is called PHOTOPHOSPHORYLATION.</td>
<td>Oxidative phosphorylation occurs in the inner membrane of mitochondria. The purpose is the same, that is, to make ATP from ADP and phosphate. This happens when electrons and hydrogens (H+) are brought along from food by the carriers to a point. As hydrogens move down the gradient across the inner membrane, the energy is harnessed to make ATP. This is called OXIDATIVE PHOSPHORYLATION. This phosphorylation is powered by redox reactions in which the electrons from food are transferred to oxygen. To sum up, oxidative phosphorylation uses energy from food, and photophosphorylation uses energy of the sunlight (photons), to produce ATP.</td>
</tr>
</tbody>
</table>

Difference between cyclic and non-cyclic

<table>
<thead>
<tr>
<th>Non-cyclic photophosphorylation</th>
<th>Cyclic photophosphorylation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electrons do not come back to the same molecule.</td>
<td>Electrons come back to the same molecule</td>
</tr>
<tr>
<td>First electron donor is water</td>
<td>First electron donor is P_{700} (PSI)</td>
</tr>
<tr>
<td>Involves both PSI &amp; PSII</td>
<td>Involves PSI only</td>
</tr>
<tr>
<td>Last electron acceptor is NADP</td>
<td>Last electron acceptor is P_{700} (PSI)</td>
</tr>
<tr>
<td>The net products are ATP, NADPH and O_2</td>
<td>The product is ATP only</td>
</tr>
</tbody>
</table>

---prepared by: Subodh Khanal---
7. Translocation of organic solutes in plants
7.1. Concept, phloem anatomy, apoplastic and symplastic transport, phloem loading and unloading
7.2. Transport mechanism: protoplasmic streaming hypothesis, contractile protein hypothesis, mass flow hypothesis, source sink hypothesis and translocation of solutes.

Translocation
The process through which the food synthesized by the leaves is reached to different plant parts is called translocation of food.

Direction of translocation-
- Downward translocation- translocation of food from leaves to stem and root of the plants.
- Upward translocation – such stages are –
  - Germination of seeds,
  - Formation of new stems and leaves from the underground stem
  - Formation and development of new buds
  - Development of fruits.
- Lateral translocation – this is performed by medullary rays.

Path of translocation-The food is translocated through vascular system of plants i.e. xylem and phloem tissue.

There are four principal pathways for translocation of materials after uptake by the roots of leaves of a plant.

a. Movement in the xylem along the transpiration stream. It allows the upward movement of organic materials in the xylem from the soil solution into foliage.
b. Through the phloem or other cells such as ray parenchyma. This is the major pathway of movement of materials applied to the leaves. Subsequently, phloem flow may take solutes up to the stem apex as easily as down to the lower parts of the plant.
c. Through the cell walls. Aqueous network through the cell walls is described as apoplast (outside protoplast). It is the principal region of the apparent free space.
d. Through the intercellular spaces. The rapid systemic permeation of gases and volatiles through the plants indicates a ready movement through the intercellular spaces.

Most of the metabolic sinks in plants are connected with the source by phloem elements in vascular strands. Sugars move from source to sink down the concentration gradients. Translocation occurs in the sieve tubes of the phloem and although other sugars and derivatives and also nitrogenous compounds may be found in the phloem exudates, the most important and general constituent is the disaccharide sucrose.

1. Anatomy of phloem

Phloem: transports food materials, usually from leaves to other parts of the plant. Phloem in angiosperms is composed of sieve tube elements, companion cells, and phloem parenchyma and phloem fibers. Gymnosperms have albuminous cells and sieve cells. They lack sieve tubes and companion cells.
• **Sieve tube** elements are also long, tube-like structures, arranged longitudinally and are associated with the companion cells. Their end walls are perforated in a sieve-like manner to form the sieve plates. A mature sieve element possesses a peripheral cytoplasm and a large vacuole but lacks a nucleus. The functions of sieve tubes are controlled by the nucleus of companion cells.

• The **companion cells** are specialized parenchymatous cells, which are closely associated with sieve tube elements. The sieve tube elements and companion cells are connected by pit fields present between their common longitudinal walls. The companion cells help in maintaining the pressure gradient in the sieve tubes.

• **Phloem parenchyma** is made up of elongated, tapering cylindrical cells which have dense cytoplasm and nucleus. The cell wall is composed of cellulose and has pits through which plasmodesmatal connections exist between the cells. The phloem parenchyma stores food material and other substances like resins, latex and mucilage. Phloem parenchyma is absent in most of the monocotyledons.

• **Phloem fibres** (bast fibres) are made up of sclerenchymatous cells. These are generally absent in the primary phloem but are found in the secondary phloem. These are much elongated, unbranched and have pointed, needle like apices. The cell wall of phloem fibres is quite thick. At maturity, these fibres lose their protoplasm and become dead. Phloem fibres of jute, flax and hemp are used commercially.

• The first formed primary phloem consists of narrow sieve tubes and is referred to as protophloem and the later formed phloem has bigger sieve tubes and is referred to as metaphloem.
CHEMICAL COMPOSITION OF TRANSLOCATE

Chemical analysis of such sieve tube exudates reveal the presence of various organic compounds like sucrose, glucose, fructose, almost all amino acids, enzymes, vitamins, phytohormones, sugar alcohols, oligo saccharides like stachyose, raffinose, etc. But more than 80% of the total translocates is sucrose. However, the glucose and fructose found in the sap are rather hydrolytic products of sucrose than the actual translocates from the source.

MECHANISM OF TRANSLOCATION- Following theories have been proposed to explain the mechanism of translocation-

Diffusion Hypothesis- according to this hypothesis, the conc. of food material is higher at their site of synthesis –the leaves and their conc. is low in roots. So, the food material diffuses to roots.

Protoplasmic streaming theory - According to Devries and Curtis soluble food materials in sieve tubes move from one end to another end due to cytoplasmic streaming.

It was purposed by Hugo de Vries (1885). Organic solutes which enter the sieve tube are possibly carried by the streaming protoplasm from one end to other of sieve tube. Organic solutes move by diffusion through the pores of sieve plate. Thus the streaming protoplasm acts as conveyer belt. Different substances move in different direction at the same time in the same sieve plate.

Objections:

- The observed rate of protoplasmic streaming is much slower than the rate of translocation.
- Protoplasmic streaming has not been observed in matured sieve plate of most plant.

Protein - Lecithin Theory:

This theory was suggested by bennet Clarke (1956). According to his theory -

i. Carrier molecule composed of protein with the phospholipids called as lecithin.
ii. There are different phospholipid groups present in membrane correspond with the number of competitive group of cations and anions.
iii. Phosphate group in the phosphatide is regarded as active centers of carrier.
iv. The ions are liberated on the inner surface of the membrane by decomposition of the lecithin by the enzyme lecithinase.
v. The regeneration of the carrier lecithin from phosphatide acid and choline takes place in presence of enzymes viz. choline acetylase and choline esterase and ATP.
vi. ATP acts as the energy source for active transport.

---prepared by: Subodh Khanal
Munch mass flow hypothesis:
Munch’s “Mass Flow” Hypothesis- Munch (1930) proposed that the soluble food material in the phloem shows mass flow. Mesophyll cells of leaves synthesize sugars due to which the OP of these cells increases resulting in absorption of water through xylem. Now, the TP of these cells also increases towards upper side and produces mass flow in the protoplasm of sieve tube towards lower side. Thus, the sugars move downwards into the roots where they are utilized during respiration and growth and their conc. lowers down. This process continues.

Objection:
- The main defect of Munch- hypothesis is that it explains only unidirectional downward flow of soluble food materials.
- Mass flow is purely physical process but phloem transport is active process and requires energy.
- Saline content and other fibrils of sieve tube reduce the speed of flow even under high pressure.
Transcellular streaming (Thaine, 1964)
The solute passes in the sieve tube in straight strands which are tubular continuous from one tube to another through sieve pores. These transcellular strands are proteinaceous in nature and acts as bidirectional translocation of solutes through rhythmic expansions. Metabolic energy is used for this purpose.

Objections:
It failed to explain the induction of ATP in sieve tube.
Such type of transcellular strands have not been observed under electron microscope.

3. Phloem loading and unloading
The process by which sugar is loaded into the sieve elements from the chloroplast of the mesophyll cells has been described by Gunning, Pate and co-workers. There are two possible parallel pathways
- The sugar may move through the symplasm, chiefly by diffusion. There are many plasmodesmata connecting the cells all the way from the mesophyll to the sieve element, so that symplastic transport of sugar is possible.
- Sugar may move from the mesophyll to the phloem by diffusing through the apoplasm or extracellular free space (cell-walls of endodermis, pericycle, xylem tracheids and vessels).
PHLOEM UNLOADING AND SINK LOADING

- Whereas phloem loading at the source is an active process (requiring energy), unloading of the phloem at the sink is generally a passive process (i.e., does not require energy).
- In general, levels of sucrose in the cytosol of the sink tissue are kept low by enzymatic hydrolysis to glucose and fructose, and by the incorporation of the resulting sugars into polysaccharides (e.g., starch).
- It has been shown, however, that the uptake of assimilates by wheat endosperm cells and legume embryos is facilitated by an energy dependent membrane process similar to that responsible for phloem loading from the apoplast, although the movement is down a concentration gradient.
- In tomato, there is evidence that suggests that the rate of assimilate import is inversely related to the sucrose content of the fruit, implying that the rate of import depends on the concentration gradient.
- Differences in rate of fruit growth have been associated with sucrose hydrolysis by acid invertase. Sucrose is also converted into glucose and fructose before entering the maize endosperm.
- The rate of assimilate uptake is also influenced by sink metabolism and physical restrictions for the movement of assimilate. In wheat and maize, rate of kernel growth is related to number of endosperm cells and the number of starch granules per cell. Position of a sink relative to other competing sinks may possibly influence rate of assimilate import in terms of proximity (to assimilate supply) and resistance of the pathway (i.e., kernels and distal tip of the maize ear tend to abort first or are frequently smaller than kernels close to the base of an ear).

Factors that control translocation:

- Light and Darkness: Though there is a general tendency for the movement of more food material towards roots than shoots, light favors greater movement of organic solutes towards shoot apex and darkness favors the opposite. Interestingly, most of the glucose synthesized by leaves during the day is stored as starch and the same is converted into sucrose at nights and transported out of the leaves.
- Temperature: Translocation is greatly affected by the change in the temperature, which means, the process is a facilitated process and it needs metabolic energy, if roots or stem tips are subjected to cold temperatures, the translocation of solutes towards them is severely inhibited. On the contrary, if the temperature is increased, the rate of translocation also increases. However very high temperature is fatal.
- Effect of Phytohormones: Growth promoting hormones like indole acetic acid, Gibberellins and cytokinin are found to accelerate the rate of translocation.
- Effect of minerals: Among all the essential nutrients, boron has been found to facilitate the movement of sucrose. The absence of boron reduces the rate of translocation significantly. Phosphate has also been implicated in this process but not much is known,
how phosphate facilitates or affects the process of translocation. Even K+ ions and other metallic ions have thought to play an important role in electro-osmosis, but their exact mechanism is not substantiated by any reasonable experiments.

- Effect of Concentration Gradient: Steeper the gradient, greater is the rate of translocation and vice versa. Such a gradient mediated movement of solutes can be demonstrated by cutting off the leaf which acts as the supply end. By removing the leaves, the gradient will be abolished and automatically the transportation comes to stand still.

**A bulk-flow mechanism translocates phloem sap from sugar sources to sugar sinks**

Translocation = The transport of the products of photosynthesis by phloem to the rest of the plant.

- In angiosperms, *sieve-tube members* are the specialized cells of phloem that function in translocation.

  => Sieve-tube members are arranged end-to-end forming tong *sieve tubes*.

  => Porous cross walls called *sieve plates* are in between the members and allow phloem to move freely along the sieve tubes.

- Phloem sap contains primarily sucrose, but also minerals, amino acids and hormones.

**Source-to-Sink Transport**

Phloem sap movement is not unidirectional; it moves through the sieve tubes from a source (production area) to a sink (use or storage area).

- **Source** = Organ where sugar is produced by photosynthesis or by the breakdown of starch (usually leaves).
- **Sink** = Organ that consumes or stores sugar (growing parts of plants, fruits, non-green stems and trunks, and others).

Sugar flows from source to sink.

- Source and sink depend on season. A tuber is the sink when stockpiling in the summer, but is the source in the spring.
- Minerals may also be transported to sinks.
- The sink is usuatty supptied by the nearest source.
- Direction of flow within a phloem etement can change, depending on locations of the source and sink.

**Translocation**

1. Movement of solutes in phloem.
   a. 90% sugar by dry weight

---prepared by: Subodh Khanal
b. remainder is amino acids, nucleotides, some hormones, potassium ions
2. Movement from **source** to **sink**.
   a. **Source** = region where solutes enter phloem.
      may be:
      i) photosynthesizing leaf
      ii) roots, tubers, bulbs, etc., that are breaking down stored starch and lipids for new
         growth (usually in spring)
      iii) seed that is germinating
   b. **sink** = region where solutes leave phloem and are used for metabolism (respiration) or
      are stored (usually as starch).
      may be:
      i) roots, tubers, bulbs, etc.
      ii) developing seeds
      iii) flowers
      iv) young, developing, leaves
      v) fruits

Sucrose is unloaded at the sink end of sieve tubes.

- In some plants, sucrose is unloaded from the phloem by active transport.
- In other species diffusion moves the sucrose from the phloem into the cells of the sink.
- Both symplastic and apoplastic routes may be involved.

**Phloem** and **xylem** are complex tissues that perform transportation of food and water in a plant.
They are the vascular tissues of the plant and together form vascular bundles. They work
together as a unit to bring about effective transportation of food, nutrients, minerals and water.

<table>
<thead>
<tr>
<th>Improve this chart</th>
<th>Phloem</th>
<th>Xylem</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Function:</strong></td>
<td>Transportation of food and nutrients from leaves to storage organs and growing parts of plant.</td>
<td>Water and mineral transport from roots to aerial parts of the plant.</td>
</tr>
<tr>
<td><strong>Movement:</strong></td>
<td>Bidirectional (Moves up or down the plant's stem from &quot;source to sink&quot;)</td>
<td>Unidirectional (Moves up the plant's stem)</td>
</tr>
<tr>
<td><strong>Additional Functions:</strong></td>
<td>Forms vascular bundles with xylem</td>
<td>Forms vascular bundles with phloem and gives mechanical strength to plant due to presence of lignified cells.</td>
</tr>
<tr>
<td><strong>Structure:</strong></td>
<td>Tubular with soft walled cells</td>
<td>Tubular with hard walled cells</td>
</tr>
<tr>
<td><strong>Elements:</strong></td>
<td>Sieve tubes, companion cells, phloem parenchyma, bast fibers, intermediary cells</td>
<td>Tracheids, vessel elements, xylem parenchyma, xylem sclerenchyma</td>
</tr>
<tr>
<td><strong>Nature of tissue:</strong></td>
<td>Living tissue</td>
<td>Non living tissue at maturity</td>
</tr>
</tbody>
</table>

---prepared by: Subodh Khanal---
8. Growth, differentiation and development

Growth is, a quantitative term, an irreversible increase in number, volume (size) or weight of cell, tissue or organ in plant life cycle.

It involves

Cell division in meristems, by increasing cell number, increases the potential for growth.
• However, it is cell expansion that accounts for the actual increase in plant mass.
• Together, these processes contribute to plant form.

Exceptions: seedling dry weight less than the seed during germination. Megaspore formation (4→1)

Development

• Development refers to the sum of all of the changes that an organism goes through in its life cycle, including growth and differentiation. Ultimately, development is an expression of the genetic program that directs the activities and interactions of individual cells.

• Leaf: leaf primordium → young → mature leaf

• Root: primordium → young → root system

• Fruit: fertilized egg → young → ripened fruit

Differentiation: The specialization of cells with the same set of genetic instructions to produce a diversity of cell types is called differentiation.

Types of Growth - Classified by Developmental Stages

Primary growth:
Apical meristems extend roots and shoots by giving rise to the primary plant body

Secondary growth:
Lateral meristems add girth by producing secondary vascular tissue and periderm

A meristem is the tissue in most plants consisting of undifferentiated cells (meristematic cells), found in zones of the plant where growth can take place.

Apical meristems

The apical meristem, or growing tip, is a completely undifferentiated meristematic tissue found in the buds and growing tips of roots in plants. Its main function is to begin growth of new cells in young seedlings at the tips of roots and shoots (forming buds, among other things). Specifically, an active apical meristem lays down a growing root or shoot behind itself, pushing itself forward.

There are two types of secondary meristems, these are also called the intercalary meristems (meant for producing growth in length) lateral meristems because they surround the established stem of a plant and cause it to grow laterally (i.e., larger in diameter).

Phases of growth

---prepared by: Subodh Khanal
Grand period of growth: The growth of tissues, organs and even a whole plant exhibit a typical model with slower-faster--slower in life cycle. The total growth appears S-shape growth curve--logistic curve and the growth rate is a parabola.

**Growth analysis**
- The growth in the base of a model--logistic curve.
- $W=W_0e^{rt}$
- $W$--weight after growth, $W_0$--initial weight, $t$--growth time, $r$--growth rate

![Growth Curve](image)

The curve can be shown appearing slowly along the line and stabilizing.

During the initial stage, i.e., during the lag phase, the rate of plant growth is slow. Rate of growth then increases rapidly during the exponential phase. After some time the growth rate slowly decreases due to limitation of nutrients. This phase constitutes the stationary phase.

**Environmental factors influencing growth**
- (1) **Light**
  - Indirectly: by photosynthesis and transpiration.
  - Directly: photomorphogenesis. *A little flux of light can control morphogenesis of the plant.*
    - Etiolation: Abnormal growth caused by light deficiency. Etiolation is characterized by thin- long stem and internodes, yellow and unexpending leaf, apical hook, poor tissue differentiation, high water content.

(2) **Temperature**--Three cardinal points:
- minimum, optimum and maximum temperature for growth
- optimum temperature for growth is the temperature at which plant grows fastest but not strongest.
• **Suboptimum temperature for growth** is the temperature at which plant grows slower but strongest than at optimum temperature.

(3). Water

• “Dry for root, wet for bud”. Root depends on cell division and bud does cell elongation.

4. O₂: enough O₂ favors growth. 10-15% soil O₂——tilling

5. Mineral nutrition: Grow worse upon deficiency or toxification

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**Germination**

**Definitions**

Seed physiologist: Protrusion of the primary root
Seed technologist: Emergence and development from the seed embryo of those essential structures which are indicative of the ability to produce a normal plant under favorable conditions

Others: Resumption of active growth of the embryo

**Germination** is the process by which plants, fungus and bacteria emerge from seeds and spores, and begin growth. The most common example of germination is the sprouting of a seedling from a seed of an angiosperm or gymnosperm. However the growth of a sporeling from a spore, for example the growth of hyphae from fungal spores, is also germination. In a more general sense, germination can imply anything expanding into greater being from a small existence or germ.

**Seed Viability**

Viability is defined as the capability of a seed to germinate and produce a normal seedling. Testing seed viability generally follows a standard procedure and is run under optimum conditions. If a germination test produces low results = low quality seed. A germination test that produces high results may not = field conditions.

**Types of Germination**

- **Epigeal Germination**: The cotyledons rise above the soil surface. Most dicots undergo epigeal germination.
- **Hypogeal Germination**: The cotyledons remain below the soil surface. All monocots have this type of germination and few dicots.

---prepared by: Subodh Khanal

Types of germination (Peffley, 1998).
Environmental conditions affecting seed germination

(1) Water: soften the seed coat, radicle elongates easily.
• O2 permeability, embryo metabolism rises.
• gel to sol activates enzymes.
• stored macromolecular substance hydrolysis
• Plant hormone from the conjugate to free form.
• 30-70% water content for starchy seed germination and more than 110% for protein seed (soybean) germination.

(2) Temperature
• Three cardinal points: “minimum, optimum and maximum”
• Optimum temperature of germination is the temperature at which seeds germinate fastest with highest germination rate.
• The larger difference (10º) between day and night favors to germination.
• The sowing should be conducted at the temperature 2-3º above the minimum temperature in production.

(3) O2
• Sufficient O2——active metabolism——growth——fast germination.
• Deficient O2——anaerobic respiration——over exhausting stored substance and alcohol toxification.
• Oil seeds (soybean, peanut, sun flower require more O2 than starchy seeds (wheat, corn), RQ<1.
• Shallow- sowing in practice.

(4) Light: light favored seed (lettuce cv. Grandrapids) germination controlled by red light (660 nm) and far-red light (730nm).
• The seeds germinate well under darkness but poorly under light.

- Water Imbibition Influenced by seed coat permeability.
- Enzyme Activation Enzymes break down stored food, aid in translocating materials and aid in synthesis of cellular structures.
- Embryo Growth Initiation Embryo is stimulated to grow.
- Seedling Establishment This occurs when roots are taking up H2O and nutrients and the shoot is undergoing photosynthesis.

Requirements for Germination
• Air (20% O2, 0.03% CO2, 80% N2)
  ✔ Oxygen most critical, required for respiration
  ✔ High levels of CO2 inhibitory
  ✔ Nitrogen no effect
• Temperature

Physiology and biochemistry of seed germination
Water uptake; seed germination starts with the imbibitions of water by dry seed coat. Various hydrophylic groups such as –NH2, -OH, -COOH of protein, polymeric carbohydrates are found in seed coat attracting the water molecules. The swelling is followed by intensive water uptake. Due to imbibitions the seed coat becomes:
More permeable to water and oxygen
Less resistance to outward growth of embryo.

- Water imbibition
  - Dependent on the composition of the seed
  - Dependent on the chemical composition of the seed
  - Proteins (“zwitterions”) cause swelling
  - Proteins > starch > lipid
  - Seed coat permeability
  - Heat killed seeds imbibe more rapidly than viable seeds
  - Greatest near micropyle/hilum
  - Muciliages enhance imbibitions

(2) Respiration: the water uptake is accompanied by rapid increase in respiration rate of embryo. Initially there may be anaerobic respiration but is soon replaced by aerobic. Respiration rate-slower -faster-slow (normal), RQ>1 during initially germinating stage being anaerobic respiration.

Increased respiration leads to increased
- ATP synthesis
- ATP synthesis permits
- subsequent growth
  A) Water uptake, O2 and germination
  B) Nucleotide levels (ADP, ATP, total nucleotides)

3. Mobilization of reserved food materials: as germination processes there is mobilization of reserved food materials to provide;
  - Building blocks for the development of embryo.
  - Energy for biosynthesis process.
  - Nucleic acids for regulation of protein synthesis and overall embryonic development.

Change in these components can be discussed as follows:
- Nucleic acids; denovo synthesis causes increase in RNA concentration producing cell division thus increasing DNA. Nuclear acid and protein synthesis. Long life mRNAs exist

---prepared by: Subodh Khanal
in mature seed and synthesize protein during germination. De novo mRNAs and proteins (hydrolyase). DNA is synthesized during the late stage.

- **Carbohydrates:** starch converts into soluble sucrose then to glucose
- **Lipids:** beta oxidation of FA to acetyl co A
- **Proteins:** de novo synthesis of peptidase and help in formation of RNA and then protein.
- **Inorganic materials:** phosphate, calcium, Mg, K are also stored in seeds these materials are also liberated.
- **Conversation for stored substance into small molecular (soluble) products for resynthesis**

![Chemical reactions and pathways diagram](image)

6. **Change in plant hormones**
   - Free IAA ↑, IAA conjugate ↓.
   - GA, Eth, CTK ↑.
   - ABA ↓.

6. Cell differentiation and cell elongation
7. Emergence of seedling and establishment
8. Hydration of seed coat

**Pattern of Germination**
- “Trigger agent” – factor that elicits germination but whose presence is not required throughout (e.g., light, temperature); shifts balance from inhibitor to promoter
- “Germination agent” – factor that must be present throughout germination (GA3)

**Sequence of events for germination**
- Radicle protrusion is the final sequence of germination
- Seedling becomes autotrophic
- Germination is ended by visible primary root growth

---prepared by: Subodh Khanal
Seed dormancy

- Seed dormancy: The mature seeds do not germinate under optimum conditions. The failure of viable seeds to germinate under suitable condition is termed as dormancy.

Types:
- Imposed dormancy: due to unfavorable condition.
- Innate dormancy: when active growth is arrested even on favorable condition.
- Secondary dormancy: many crops capable of germinating immediately after ripening but pass to dormant phase this type is called as secondary dormancy.

Categories of seed dormancy:
- Seed coat:
  1. Hard seededness: Seed fail to imbibe water due to impermeable seed coat.
  2. Mechanical resistance: Rigid seed coverings resist embryo expansion even though the tissues may successfully take up water.
  3. Chemical inhibitors: Seed coverings contain chemicals which inhibit germination
  Morphologically undeveloped embryo (Rudimentary embryo):
  Some species produce seeds with extremely small and underdeveloped embryos in seeds that are mature enough to detach from the plant. Such seeds simply need much more time for the embryo to grow and develop within the seed prior to germination.

- Internal dormancy:
  1. Physiologically shallow dormancy: Present in some freshly harvested seed. Disappears with dry storage over a period of days or months.
  2. Light dormancy: Some species have exact requirements of light quality, quantity, and duration to induce germination.
  3. Temperature dormancy: Many seeds are specific in their temperature requirements. Some need alternating temperatures (day/night). Others germinate over a narrow range of temperatures and go dormant at temperatures above or below this range.
  4. Physiologically deep dormancy: Seeds are unable to germinate until they undergo a cool-moist period over a few days or a few months.
  Combined dormancy: More than one type of dormancy may occur within the same seed.
  Induced or secondary dormancy: Viable, non-dormant seeds may become dormant when placed in environments which are unfavorable to germination.

- Exogenous dormancy

Exogenous dormancy is caused by conditions outside the embryo and is often broken down into three subgroups:

- Physical dormancy: Occurs when seeds are impermeable to water or the exchange of gases. Legumes are typical examples of physically dormant seeds; they have low moisture content and are prevented from imbibing water by the seed coat.
Mechanical dormancy: Mechanical dormancy occurs when seed coats or other coverings are too hard to allow the embryo to expand during germination. In the past this mechanism of dormancy was ascribed to a number of species that have been found to have endogenous factors for their dormancy instead. These endogenous facts include physiologically dormancy cased by low embryo growth potential.

Chemical dormancy: Includes growth regulators etc., that are present in the coverings around the embryo. They may be leached out of the tissues by washing or soaking the seed, or deactivated by other means. Other chemicals that prevent germination are washed out of the seeds by rainwater or snow melt.

Endogenous dormancy

Endogenous dormancy is caused by conditions within the embryo itself, and it is also often broken down into three subgroups: physiological dormancy, morphological dormancy and combined dormancy, each of these groups may also have subgroups.

Physiological dormancy: Physiological dormancy prevents embryo growth and seed germination until chemical changes occur. These chemicals include inhibitors that often retard embryo growth to the point where it is not strong enough to break through the seed coat or other tissues. Physiological dormancy is indicated when an increase in germination rate occurs after an application of gibberellic acid (GA3) or after Dry after-ripening or dry storage. It is also indicated when dormant seed embryos are excised and produce healthy seedlings: or when up to 3 months of cold (0–10°C) or warm (=15°C) stratification increases germination: or when dry after-ripening shortens the cold stratification period required. In some seeds physiological dormancy is indicated when scarification increases germination.

Seeds with physiological dormancy most often do not germinate even after the seed coat or other structures that interfere with embryo growth are removed. Conditions that affect physiological dormancy of seeds include:

- **Drying**;
- **Photodormancy**
- **Thermodormancy**
- Morphological dormancy: Embryo underdeveloped or undifferentiated. Some seeds have fully differentiated embryos that need to grow more before seed germination, or the embryos are not differentiated into different tissues at the time of fruit ripening.
- Combinational dormancy: Combinational dormancy occurs in some seeds, where dormancy is caused by both exogenous (physical) and endogenous (physiological) conditions. Some Iris species have both hard impermeable seeds coats and physiological dormancy.
Secondary dormancy

Secondary dormancy occurs in some non-dormant and post-dormant seeds that are exposed to conditions that are not favorable for germination, like high temperatures. It is caused by conditions that occur after the seed has been dispersed. The mechanisms of secondary dormancy are not yet fully understood but might involve the loss of sensitivity in receptors in the plasma membrane.

Reasons for seed dormancy and methods to break them

1. Coat (pericarp) barrier: water cannot penetrate coat of hard seeds in legumes etc. O2 cannot penetrate coat of cocklebur etc. a strong coat, such as peach or plum nuclei (seeds) prevents embryo sprout out.
   • Methods to break: removing or wounding coats by mechanic means, high concentration of acids, hot water.
2. After-ripening limited: Not well developed embryo: ginkgo, panax, Fraxinus excelsior etc. Not ripening in physiology Wheat seed, apple seed and pine seed etc.
   Methods: Dry under full sun light, GA treatment and scarification.
3. Inhibitors in seeds
   • ABA, alkaloid, phenols and quinones.
   • Methods: Washing, GA treatment

Seed Scarification

Seed scarification involves breaking, scratching, or softening the seed coat so that water can enter and begin the germination process. There are several methods of scarifying seeds. In acid scarification, seeds are put in a glass container and covered with concentrated sulfuric acid. The seeds are gently stirred and allowed to soak from 10 minutes to several hours, depending on the hardness of the seed coat. When the seed coat has become thin, the seeds can be removed, washed, and planted. Another scarification method is mechanical. Seeds are filed with a metal file, rubbed with sandpaper, or cracked with a hammer to weaken the seed coat. Hot water scarification involves putting the seed into hot water (170 to 212°F). The seeds are allowed to soak in the water, as it cools, for 12 to 24 hours before being planted. A fourth method is one of warm, moist scarification. In this case, seeds are stored in nonsterile, warm, damp containers where the seed coat will be broken down by decay over several months.

Seed Stratification

Seeds of some fall-ripening trees and shrubs of the temperate zone will not germinate unless chilled underground as they overwinter. This so called “after-ripening” may be accomplished artificially by a practice called stratification.

The following procedure is usually successful. Put damp sand or vermiculite in a clay pot to about 1 inch from the top. Remove the fleshy outer coating (fruit) from the seed, if present. Place the seeds on top of the medium and cover with 1/2 inch of damp sand or vermiculite. Place the pot containing the moist medium and seeds in a plastic bag and seal. Place the bag in a refrigera-
tor. Periodically check to see that the medium is moist, but not wet. Additional water will probably not be necessary. After 10 to 12 weeks, remove the bag from the refrigerator. Take the pot out and set it in a warm place in the house. Water often enough to keep the medium moist. Soon the seedlings should emerge. When the young plants are about 3 inches tall, transplant them into pots to grow until they are ready to be set outside.

**Other method are:**
- Chilling treatment; low temperature(0-5°C) for some time e.g. apple, peach, plum
- Alternate high and low temperature: 30-40°C to 5-10°C. Care to be taken that difference should not be more than 20°C. e.g. Rumex, Lycopus
- Impaction: by vigorous shaking for seeds having plug for easy breaking.
- Light requirement: red light hasten germination in positive photoblastic seeds
- Pressure treatment; high hydraulic pressure about 2000 atm at temperature of 18-20 ºC for 5-20 minutes. E.g. sweet clover, alfa alfa
- Chemical treatment: thio urea, KNO3, 2- chlorohydrin, purine, gibberlin, cytokinine hasten the germination in several plants.

**Photoperiodism** is the physiological reaction of organisms to the length of day or night. It occurs in plants and animals. Photoperiodism can also be defined as the developmental responses of plants to the relative lengths of the light and dark periods. Here it should be emphasized that photoperiodic effects relate directly to the timing of both the light and dark periods.

In 1920, W. W. Garner and H. A. Allard published their discoveries on photoperiodism and felt it was the length of daylight was critical, but it was later discovered that the length of the night was the controlling factor. Photoperiodic flowering plants are classified as long-day plants or short-day plants, even though night is the critical factor, because of the initial misunderstanding about daylight being the controlling factor. Each plant has a different length critical photoperiod, or critical night length.

**Long-day plants**

A long-day plant flowers when the day length exceeds their critical photoperiod. These plants typically flower in the northern hemisphere during late spring or early summer as days are getting longer. Some long-day obligate plants are:

- Carnation (Dianthus)
- Henbane (Hyoscyamus)
- Oat (Avena)

Some long-day facultative plants are:

- Pea (Pisum sativum)
- Barley (Hordeum vulgare)
- Lettuce (Lactuca sativa)
- Wheat (Triticum aestivum, spring wheat cultivars)
- Turnip (Brassica rapa)

Short-day plants flower when the day lengths are less than their critical photoperiod. They cannot flower under long days or if a pulse of artificial light is shone on the plant for several minutes during the middle of the night; they require a consolidated period of darkness before floral development can begin. Natural nighttime light, such as moonlight or lightning, is not of sufficient brightness or duration to interrupt flowering.

Some short-day obligate plants are:

- Chrysanthemum
- Coffee
- Poinsettia

**Day-neutral plants**

Day-neutral plants, such as cucumbers, roses and tomatoes, do not initiate flowering based on photoperiodism at all; they flower regardless of the night length. They may initiate flowering after attaining a certain overall developmental stage or age, or in response to alternative environmental stimuli, such as vernalisation (a period of low temperature), rather than in response to photoperiod.

**Short long day plants**: these are long day plants but must be exposed to short day in early periods of growth for subsequent flowering. E.g. rye, wheat

**Long short day plants**: these are short day plants but must be exposed to long day during early period for subsequent flowering e.g. Bryophyllum, night jasmine.

**Intermediate plants** (sterophotoperiods) : these plants can only flower within a definite range of light hours above and below where no blossoming occurs. E.g. some varieties of sugarcane

**Amphiphotoperiodic plant**: they behave just opposite to the intermediate plants where they remain vegetative in intermediate day length e.g. Mediaelgens.

**Critical day length**

The period of daylight, specific in length for any given species, that appears to initiate flowering in *long-day plants* or inhibit flowering in *short-day plants*. In actual fact long-day plants will not flower if the dark period exceeds a certain maximum and conversely short-day plants will not flower unless the dark period exceeds a certain minimum. These periods are termed critical dark periods and must be continuous to have effect

**Importance of photoperiodism**

- It has a great application in hybridization programme.
- Useful for successive cultivation of agricultural crops.
- The yield of tubers, bulbs, rhizomes, corms can be increased by the knowledge of photoperiodism.

---prepared by: Subodh Khanal
The vegetable can be made to remain vegetative for longer periods and annuals can be growth 2-3 times a year.

Long day photoperiodic induction influences stolen formation in strawberries.

Short day photoperiodic induction help in production of winter buds, thickening of radish, carrot, dahlia

Winter dormancy and autumn leaf fall can be prevented by increasing photoperiodic hours.

It serves to stimulate the development of fruit color, flower and fruit formation throughout the year.

Physiological preconditioning activities can be induced by the treatment of photoperiods.

It probably may be of direct importance in the synthesis of food materials as well as hormones which are almost essential for better growth and development of crops.

**Mechanism of photoperiodism**

**Phytochrome theory**

- Phytochrome is a homodimer: two identical protein molecules each conjugated to a light-absorbing molecule (compare rhodopsin).
- Plants make 5 phytochromes: **PhyA**, **PhyB**, as well as C, D, and E.
- Plants make such adjustments by utilizing the pigment phytochrome, which exists in two forms: **P_r**, which absorbs red light, and **P_fr**, which absorbs far-red light. Each can convert to the other when they absorb light. During the day, the two forms convert back and forth (**P_r** becomes **P_fr**, and vice versa), until they reach an equilibrium of 60:40 **P_fr**: **P_r** in plant tissues. During the night, **P_fr** slowly converts to **P_r** or else disintegrates. **P_r** is stable in the dark.
- Phytochromes exist in two interconvertible forms
  - **P_r** because it absorbs red (**R; 660 nm**) light;
  - **P_fr** because it absorbs far red (**FR; 730 nm**) light.
- These are the relationships:
  - Absorption of red light by **P_r** converts it into **P_fr**.
  - Absorption of far red light by **P_fr** converts it into **P_r**.
  - In the dark, **P_fr** spontaneously converts back to **P_r**.

---prepared by: Subodh Khanal
- Pfr+Pr no flowering in SDP but promotes in LDP.

**Hormonal theory (Long, Brain, Naylor)**

According to this theory CO₂ produce a precursor and leads to gibberlin like hormone during appropriate light condition. During night it gets converted back to the precursor. Pr and Pfr play a role in the formation of gibberlin like hormone for flower induction.

\[
\begin{align*}
\text{Pr} & \rightarrow \text{CO}_2 \rightarrow \text{precursor} \leftrightarrow \text{GA like hormone} \rightarrow \text{flower induction} \\
\text{Pfr} &
\end{align*}
\]

**Florigen**

- Because hormones control so many metabolic activities in plants, flowering has long seemed likely to be under the control of one or more hormones. Early experiments sought to determine which part of a plant is sensitive to the light that initiates flowering.
- The results suggested the presence of a substance that moved from the leaves to the flower buds. Although the substance was not identified then—nor has it been isolated now—it was named florigen.
- Florigen is the hypothetical flowering hormone; it may or may not actually exist. Note that flowering most likely is not controlled by a single hormone, but is the result of a combination of internal and external signals and responses.

**Theory of endogenesus rthythm**

- Photophilous phase: light is essential. Anabolism is dominant and catabolism is less or weak hence stimulates the initiation of flowering.
- Scotophiulous phase: dark is essential where the catabolism is dominant and anabolism is weak or less hence inhibiting flowering.
**Vernalization** (from Latin: vernus, of the spring) is the acquisition of a plant's ability to flower or germinate in the spring by exposure to the prolonged cold of winter. After vernalization, plants have acquired the ability to flower, but they may require additional seasonal cues or weeks of growth before they will actually flower.

The promotion of flowering in response to a prolonged exposure to cold temperatures (i.e. winter) is a useful adaptation for plant species that flower in the spring. This promotion is known as vernalization. Chouard (1960) defined vernalization as the acquisition or acceleration of the ability to flower by a chilling treatment. Thus, a vernalizing cold treatment does not initiate flower primordia directly, but creates the capacity for subsequent flowering. Vernalization does not refer to the breaking of dormancy by cold, such as the release of pre-formed floral buds after chilling or the promotion of seed germination by cold (stratification). The vernalization response can be facultative or obligate. Winter annuals, for example have a facultative vernalization response: cold exposure is not required for flowering, but flowering will occur more rapidly after cold treatment. Biennials in contrast, have an obligate requirement for cold treatment and thus cannot flower without prior cold exposure.

In most species the effect of a vernalizing cold treatment can be partially or totally eliminated by several days of heat treatment, typically 30-40°C (devernalization). The heat treatment, however, must be applied immediately after cold treatment: after several days at normal growth temperatures devernalization is ineffective. Furthermore, the ability of heat treatment to ‘devernalize’ declines with increased duration of cold treatment. It is interesting to note that in some plants, such as Arabidopsis and certain varieties of Chrysanthemum, devernalization is only effective if the plants have been cold treated in darkness (reviewed in Bemier et al. 1981). This may reflect a requirement for a threshold level of metabolic activity or cell division in order for the vernalized state to become fixed.

**Age and Site of Vernalization:**

- Vernalization through cold treatment is very effective at the seed stage or seedling stage.
- In some cereals, even the embryos can be successfully verbalized. However, in many cold recurring species, vernalization is not effective until and unless the plant possess at least few leaves.
- The requirement of few leaves for effective vernalization is called ‘Ripeness to Flowering’. This suggests that plants need certain degree of photosynthetic obtain to respond for cold treatment.
- Probably the role of carbohydrates in vernalization is to supply some energy. Nevertheless, the most sensitive site which acts as the perceptive organ is the meristematic region of the shoot apex.

**Temperature Effect:**

- For the normal growth and development, every plant requires an optimum temperature. But for vernalization the optimum temperature required is 3°C to 17°C, which varies depending upon the species involved.
However the efficiency of cold treatment in bringing about vernalization is determined by the number of days shortened between germination and flowering stage.

**Effect of water and oxygen:**

- Along with the cold treatment plants also require water and oxygen for effective vernalization.
- The seeds or embryos should possess at least 40-50% water in their cells, without which cold treatment has no effect.
- Similarly oxygen is very essential; probably it is required for biological oxidation. Still, it is difficult to explain how cells use carbohydrates and oxygen for enzymatic oxidative process at such low temperature.

**Vernalin:**

- The presence of such substance has been demonstrated by grafting a vernalized plant to another non vernalized plant at normal temperatures.
- Some substance is synthesized and such substance is now called ‘Vernalin” and it is capable of diffusion. Attempts to isolate and identify the components of vernalin have failed. Whether the vernalin is the same as florigin or a precursor of florigin is not known.

**Devernalization:**

- If vernalized seedlings or seeds are subjected to higher temperature like 35-40 °C the plants that develop from such treatment fail to flowers. Such a nullifying effect by higher temperatures is called Devernalization.
- Nevertheless, if the vernalized plants are maintained at sufficiently low temperatures for a long period of time, which has to be determined for every species, devernalization is not possible. This may be due to the putative vernalin have already acted upon the genetic material and committed it is flower formation.
- However, devernalized plants can be revernalized by subjecting the same seedling or seed again for another period of cold treatment by repetition of vernalization and devernalization cycles. Prolonged vernalization the effect decreases and seedlings loose their viability and potentiality to produce flowers.

**Mechanism of floral induction in vernalized plants:**

- It is clear from the earlier discussions that the plants with their specific genetic make up respond to different treatments like cold or photoperiods and produce flowers.
- Most of the cold requiring plants also require proper photoperiodic treatment. Gibberellins are known to overcome both cold treatment and photoperiodic treatment in long day plants, but it has no effect on short day plants. Synthesis of some unknown
substance called vernalin during the period vernalization has been clearly demonstrated by grafting experiments.

- Furthermore for proper vernalization, plants require sufficient amount of water, oxygen and some vegetation growth.
- Though all the above said factors are provided to the plant, flower inducing substance won’t be synthesized until and unless it is treated with proper cold condition at the stage of its development. It is during the cold treatment, the synthesis of the said flowering inducing factor is believed to be accelerated.

According to the hypothesis proposed by Purvis et.al. (older view), plants normally synthesize a substance called ‘B’ from some unknown precursor called ‘A’. The synthesis of B is accelerated by cold treatment, when ‘B’ is synthesized; it persists for a period of time. Further conversion of B to C and then D is under the control of photoperiods.

Chailkhyan, on the other hand is of the view that floral induction requires two substances. One is GA or GA like compound and the other is flower inducing substance. So he assumed that cold requiring long day plants contain enough flowering substance but lack in GA like compound which will be synthesized under inductive conditions. Contrary to this, cold requiring short day plants possess sufficient amount of GA like compounds and lack in flowering substances which will be synthesized during inductive conditions. The GA like compound and flowering substance combine to produce a flower inducing complex called florigen. Whether it is a cold requiring plant or photoperiod requiring plant or a plant which requires both, the hormone that is required for flowering has to be the same. Chailkhgan’s view was supported by G. Melcher, who grafted a short day Maryland mammoth plant (it does not require cold treatment), to another non–verbalized cold requiring long day Hyoscyamus plant. Grafting resulted in the formation of flowers in Hyoscyamus plant even under non inductive conditions.

Importance

- Vernalization activates a plant hormone called florigen present in the leaves which induces flowering at the end of the chilling treatment.
- Crops can be produced earlier and can also be produced in the region where they are not naturally grown or produced.
- Plant breeding programme can be accelerated with its knowledge.
- It increases the cold and drought resistance in plants.
- It helps to reduce disease incidence in several plants.
- It helps to improve yield and remove wrinkledness of kernels and grains.
- Several seeds of crop plants, bulbs and buds of temperate species requires vernalization.
- Offseason flower and vegetable can be produced with its application.
- It may resolve the adverse condition during the period of development of crops.
- Devernalization helped to control flowering in onion, garlic, potato and other plants.
- It shortens the vegetative period and hasten flowering period which will benefit to the farmers.
- Some plant species do not flower without vernalization. Many biennial species have a vernalization period, which can vary in period and temperature. Typical vernalization temperatures are between 5 and 10 degrees Celsius (40 and 50 degrees Fahrenheit)
9. **Plant Growth Regulators**

- Certain substances affect the growth quite miraculously. These were referred to Hormones. Hormone means to urge or to stimulate (Greek word).
- Hormones of plants are referred as phyto Hormones. Phyto Hormones are organic substances which are naturally produced in plants; control the growth or other physiological functions, at a sight remote from its place of production and active in extreme minute quantities.
- Growth Hormones can also be defined as substances which are naturally produced in plants, control the growth are other physiological functions, at a sight remote form its place of production and active in extreme minute quantities.
- Growth Hormones can also be defined as substances synthesised in particular cells and are transferred to other cells where in extremely small quantities influence development process.

According to Devlin and Witham (1986), “*plant growth regulators* are organic compounds other than nutrients that in small amounts promote, inhibit, or otherwise modify a physiological process in plants. Plant hormones or phytohormones are regulators produced by plants, in which a low concentration regulates plant physiological processes.” (p. 354). The growth regulators can either be natural or synthetic and are widely being used in the agricultural production and even for economic importance.

**Plant growth regulators/ Plant hormones/ Phytohormones**

Plant hormones and growth regulators are chemicals that affect flowering, aging, root growth, distortion and killing of leaves, stems, and other parts; prevention or promotion of stem elongation, etc.

A plant growth regulator is also an organic compound, either natural or synthetic, that modifies or controls one or more specific physiological processes within a plant through translocation. And they can be either synthetic or natural. The growth regulators include:

1. **Auxins**
2. **Gibberellins**
3. **Cytokinins**
4. **Ethylene**
5. **Abscisic acid**
6. Flowering hormones
7. Florigen, Anthesins, Vernalin,
### Classification of PGR

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---prepared by: Subodh Khanal
Miscellaneous Natural Substances | Vitamins, Phytochrome Transmissive Substances  
--- | ---  
Synthetic Growth Retardents | Ccc, Amo, 1618, Phosphin - D, Morphacting, Malformis.  
Miscellaneous Synthetic Substances | Synthetic Auxins, Synthetic Cytokinins.

**Auxins**: from Greek *auxein*, “to increase”)
Chemically, auxin is indoleacetic acid (IAA), which is synthesized from indole or tryptophan. There are a number of synthetic “auxins” too. Auxins promote growth in molar concentrations of $10^{-3}$ to $10^{-8}$.

![IAA and NAA structures](image)


**Role of Auxins**

- Apical Dominance: Removal of apical bud stimulates lateral buds. Auxins inhibit lateral bud formation since they are synthesized in apex. This phenomenon is called apical dominance. Eg: Potato tubers for apical buds forming.
- Cell Division and Elongation: Shoot and Root growth.
- Xylem Differentiation: Auxins helps in establishing contact between vascular tissues of the callus and that of the bud and makes it possible for the bud to grow properly in callus. By adding Auxins and sugar continued growth of callus may be obtained and new shoots and even new plant can be produced.
- Nucleic Acid Activities of IAA increases total RNA - synthesizes specific enzymes lead to cell enlargement.
- Manifold Activities Play specific role in seed germination, growth, rooting, flowering (Reproductive phase), abscission, parthenocarpy and tissue culture.

**Practical Applications of Auxins**

- Germination: IAA, IBA, NBA, 2,4-D are mostly used in soaking seed for germination- at low concentrations promotes germination but these effects are subjected to variation depending on form and species of plants.
- Root: NAA, 10% induces 100% rooting in mango: Dashri, langra IBA+SUGAR application leads to greater number of roots-structure of roots also changed (Vascular bundles).

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Flowering: Play floragenic role in day neutral plants IAA promotes formation of female flowers. Increased spikelet number, leaf number and weight and number of grains in wheat. NAA & IAA increases boll-set (G.hirsutum) induced more pine-apple. Fruit weight increases.

Parthenocarpy: IBA, NAA produces seed less/fruits - smaller sized fruits, but more in number, hence yield not affected.

Fruit setting: By using 2,4,5 T fruit setting and yield of ber/fruit increased. IAA, IBA, and NAA induce high percentage fruit set.

Prevention of pre-mature drop of fruits: 2,4,D,IAA,IBA, 2,4,5-T, are used to prevent pre-harvest drop of sweet oranges( 100 to 500 ppm)

Tissue and Organ culture: IAA & Kinetin

Auxins as inhibitors: High concentration of auxins inhibit the growth and exert toxic effect on plants. In normal case, self produced auxins inhibit the growth and development of lateral buds, and as a result apical buds, remains dormant.

**Auxins use in Agriculture and Horticulture**

- Propagation of plants by hormone treatment of cuttings
- Prevention of pre harvest drops of plants.
- Increasing parthenocarpy.
- Increasing fruit set.
- Prevention of sprouting by inhibiting buds.
- Inhibition of prolonged dormancy.
- Control of flowering.
- Defoliation of plants
- Prevention of leaf fall or abscission.
- Thinning of compact fruits.
- Selective weed killer.

**There are many different natural and synthetic auxins with different functions.**

- Indole acetic acid-IAA
- Indolebutyric acid=IBA
- Naphthalene acetic acid-NAA
- 2,4-dichlorophenoxy acetic acid-2,4-D

**Gibberellins**

was first known by a Japanese farmer Konishi (1898) and working was found out by Kurosawa in 1926. He discovered as GA. It was first extracted from the ascomycetous fungus *GibberellaFujikuroi*, the casual organisms of “foolish seedling of rice” or commonly called *bakanae of rice*. The infected plants were taller, seedless and pale in color. These symptoms were produced due the substances secreted by the fungus. It is mainly of gibberrellin $\text{GA}_3$ or $\text{A}_3$. 

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Second important growth Hormone. More than 60 types of Gibberellins are known. They are named as GA1, GA2------ up to GA60. About 51 types are found in higher plants.

Mechanism of Gibberellins: GA exerts its physiological effect on altering the Auxin status of tissue. It acts at the gene level to cause depressions of specific gene.

The activated genes by producing new enzymes bring about observed morphologic changes. Alerts the RNA. GA appears to involve in alteration of nucleic acid directed protein synthesis in some long term regulatory action and some other types of activation phenomena in short term regulatory action.

Role of Endogenous Gibberellins

- Apical bud dormancy
- Role in sub apical meristem
- Cell elongation
- Fruit growth
- Flowering
- Metabolisation of food in seed storage cells.

Practical Applications of Gibberellins

- Germination: Increases length of hypocotyls and cotyledenary leaf area.
- Root Growth: Inhibits root growth
- Leaf Expansion: Leaves become broader and enlarged (Cabbage, Sweet corn).
- Hypnoesity of leaves: GA treated leaves of chrysanthemum plants holds their leaves more erect.
- Flowering: Induces flowering in long day plants and in plants requiring cold induction. Also promotes formation of male flowers.
- Parthenocorpy: Brinjal, Guava(Alahabad round). Thomson seedless
- Fruit setting: Increased fruit setting (Phalsa, Sweet lime, Grapes).
- Fruit Drop: Not much effective.
- Stem elongation: Chorchorus capsularis: extention of stem and increased number of internodes. However leaf area, basal diameter of stem and fibre quality are reduced.
- Pollen Germination: Sugar cane 15 out of 34 germinated against normal conditions.
- Breaking Dormancy: In temperate plants buds become dormant in later summer and do not sprout even when exposed to sufficient moisture, temperature and oxygen. They require low temperatures or long days or red light. GA overcomes this dormancy. Enhanced cell elongation push through the endosperm (seed coat) Potato tubers can be made to sprout in winter by GA.
Other uses: Sprayed on Fruits to prevent rind disorder. Thomson seedless grape bunches if sprayed with GA, causes elongation of bunch, so they are less tightly packed and less susceptible to fungi.

GAs is used to increase the size of Thompson grapes berries. It is used by some breweries to increase the rate of malting by increasing the potential of starch digestion. Gibberellins are also used to increase sugar cane growth and sugar yields. It poses great uses in increasing stalk length and production, breaking of dormancy, inducing uniform crop emergence, producing staminate flowers, loosening of fruit clusters, increasing the fruit size, quickening of maturity, improving fruit quality, controlling cracking of fruits, reducing non-productive period, promoting vegetative growth and flower size, increasing sugar content in sugar cane, inducing flowering and so on.

Cytokinin

Skoog (1964) noted that in general “the term Cytokinin is universally used for substance which promotes cell division and exerts other growth regulatory functions as kinetin”. It also promotes cell division, and influence cell differentiation and aging of leaves. Cytokinin are a group of phenyl urea derivatives of adenine, one of the purines found in nucleotides. The first cytokinin was chemically isolated in 1913 and cytokinins were studied using coconut endosperm for a number of years starting in the 1940’s. This isolate was shown to be a potent growth promoter and was used in tissue culture and embryo development studies.

Synthetic cytokinin

Action and application

- Cell division
- Cell enlargement
- Morphogenesis
- Dormancy
- Apical dominance
- Mobility: Immobile obstructs the movement of amino acid, phosphate and various other substances
- Nucleic acid metabolism: Quick increase in the amount of RNA and decreases DNA
- Protein synthesis: Increases DNA
- Protein synthesis: Increased rate

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Florigens: Induction of flowering in short day plants.

**Role of Cytokinins** - The following are the roles and functions of gibberellins as cited by Davies, 1995; Mauseth, 1991; Raven, 1992; Salisbury and Ross, 1992:

- Stimulates cell division.
- Stimulates morphogenesis - shoot initiation/bud formation in tissue culture.
- Stimulates the growth of lateral buds - release of apical dominance.
- Stimulates leaf expansion resulting from cell enlargement.
- May enhance stomata opening in some species.

The major use for cytokinins derives from its ability to delay senescence and maintain greenness. The artificial, highly active cytokinin, benzyladenine, is the main compound used. The treatment of holly for festive decorations enables its harvest many weeks prior to use. Post-harvest sprays are now available to prolong the storage life of green vegetables such as asparagus, broccoli, and celery. These hormones also have used to increase shelf life of fruits, quickening of root induction and production of efficient root system. It is also used to increase the yield and oil contents in ground nuts, to break the dormancy, delay senescence, causes cell division and so on.

**Abscisic Acid (ABA)**

Abscisic acid (ABA) is a hormone that functions by inhibiting growth activities in times of environmental stress rather than by promoting growth. It often serves as an antagonist to the other growth promoting hormones in plants. Abscisic acid, which is also synthesized from mevalonic acid, got its name from the erroneous belief that it promoted the formation of abscission layers in leaves and fruits. It does not, although leaf abscission accompanies dormancy in many plants. It is regarded as one of the principal accelerators of abscission and also bud dormancy. It is natural growth inhibitors, and acts as a stress hormone that helps to cope with adverse environmental conditions.

![Abscisic acid](image)

**Role of ABA**

- Induces bud dormancy
- Promotes senescence
- Accelerates leaf abscission in cotton plant

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- Induces flowering during long days in certain short day plants - ineffective in short day plants.
- Counteracts GA

A list of some of the known physiological effects caused by cytokinins is listed below. (Davies, 1995; Mauseth, 1991; Raven, 1992; Salisbury and Ross, 1992).

- Stimulates the closure of stomata-water stress brings about an increase in ABA synthesis.
- Inhibits shoot growth but will not have as much affect on roots or may even promote growth of roots.
-Induces seeds to synthesize storage proteins.
- Inhibits the affect of gibberellins on stimulating.
- Has some effect on induction and maintenance of dormancy.
-Induces gene transcription especially for proteinase inhibitors in response to wounding which may explain an apparent role in pathogen defense.

**Ethylene**

The only ripening hormone. Denny as cited in Pandey and Sinha (2006) reported that ethylene is highly effective in fruits ripening. It is actually the natural product of ripening fruits. It is a gas at temperature under which a plant can survive. It is a powerful inhibitor of root and stem elongation.

![Ethylene structure](image)

- Highly useful in inducing fruit ripening. Ethylene is a natural product of ripening fruit. Ethylene is a gas at temperatures under which a plant can live.
- Auxins increase ethylene level in plants and auxin actions are attributed through ethylene such as increased percentage of female flowers, apical bud dominance and leaf epinasty.

**Role of Ethylene**

- Abscission: Principle accelerator of abscission - Capable of promoting changes associated with pre - abscission and aging of leaves, petioles, flowers and fruits. Ethylene degreening is a commercial practice (5-10 ppm).
- Degreening occurs after ethylene treated are exposed to air - accelerates maturity and induces uniform ripening (Pine apple).

**Practical Application and its economic importance** - Ethylene gas a wide variety of uses, but its gaseous nature precludes its applications in non-enclosed spaces. Ethylene itself can be used to enhance the ripening of fruits such as bananas in storage following their shipment in an unripe condition. An ethylene-producing liquid chemical, 2-chloroethylphosphonic acid - commercially called Ethrel or Ethephon has been introduced and it is sprayed onto the plant at a slightly acid pH. When it enters the cells and encounters the cytoplasm at about neutral pH, it breaks down to
release gaseous ethylene which is commercially used for fruit ripening. These hormones have a potential area of use in a wide variety of fruits such as grapes, cherries, and citrus. Promotion of female flower production in cucurbits - cucumber, squash, and melon so as to increase the number of fruits produced per plant. It is also used for the promotion of flower initiation and controlled ripening in pineapples.

**Other hormones**

- **Florigen**: These hormones are synthesized in the older leaves and then transferred to the growing region where it initiates the floral and bud initiation. It is mainly responsible for flowering in plants. It also called flowering hormones
- **Anthesins**: Its newly discovered hormones responsible for flower formation which horticulturists use to induce the early flowering in some plants.
- **Vernalin**: It is used to undergo and bring nervalisation in some plants.
- **Morphactins**: They are synthetic growth regulators which have the various roles and functions as natural growth regulators. It has been effective in flowering stimulation, sequence of flowering, position and number of flowers, formation of flowers, inflorescence parthenocarpy, etc.
- **Brassinosteroids**, are a class of polyhydroxysteroids, a group of plant growth regulators. Brassinosteroids have been recognized as a sixth class of plant hormones, which stimulate cell elongation and division, gravitropism, resistance to stress, and xylem differentiation. They inhibit root growth and leaf abscission. Brassinolide was the first identified brassinosteroid and was isolated from organic extracts of rapeseed (*Brassica napus*) pollen in 1970.
- **Salicylic acid** — activates genes in some plants that produce chemicals that aid in the defense against pathogenic invaders.
- **Jasmonates** — are produced from fatty acids and seem to promote the production of defense proteins that are used to fend off invading organisms. They are believed to also have a role in seed germination, and affect the storage of protein in seeds, and seem to affect root growth.
- **Plant peptide hormones** — encompasses all small secreted peptides that are involved in cell-to-cell signaling. These small peptide hormones play crucial roles in plant growth and development, including defense mechanisms, the control of cell division and expansion, and pollen self-incompatibility.
- **Polyamines** — are strongly basic molecules with low molecular weight that have been found in all organisms studied thus far. They are essential for plant growth and development and affect the process of mitosis and meiosis.

**Conclusion** - Growth regulators in minute quantity can have adverse effects to promote & modify physiological process in plants. The growth regulators are used: to increase the fruit sizes, to induce early flowering and increase the number of flowering; to break the dormancy of some seeds, to quicken the maturity and improve the fruit quality; to increase the sugar content in sugarcane; to increase the yield and oil contents; to promote the flower initiation and control ripening some other fruits and many more. Beside their major roles there are many other minor roles as sequence of flowering, position and number of flowers, formation of flowers, parthenocarpy, root and shoot initiation, etc. The knowledge of these growth regulators have been advantages and boon to many agriculturists and horticulturists globally.

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10. Physiological parameters influencing the productivity of crop plants

Crop production is the system of harvesting energy from sun as food, fiber, feed, drugs e.t.c and its efficiency should be assessed in terms of conversion of solar radiation into useful products in terms of yield/ area/year. This yield achieved by the result of environmental factors acting on physiological processes. Maximum yield is the success sign of crop production. To determine the efficiency of plant is converting solar energy into chemical energy and it is necessary to measure how much plant material is produced in the unit time on unit area.

The efficiency of energy conversion \( (E) = \text{calories value of plant material/solar energy available} \)

The crop productivity is determined by many methods

1. Growth characters
2. Nutrient content
3. Developmental characters.

Cereals:

1. Tillers: poor tillers determine the low yield whereas maximum and effective tillers influence the productivity of crop plants. Maximum number of tillers /sq. m is correlated with the number of ear formation phase.

2. Stem: stout, erect, plant height, number of plant population and plant density are the character to influence the productivity of crop plants. Translocation of assimilates, conduction and distribution of assimilates are mechanism of loading and unloading of phloem via stems. Sink and source is one of the vital physiological parameters to determine the yield components.

3. Canopy structures; shape, size, length and arrangement of leaves are the most effective measures of crop productivity. The rate of crop photosynthesis depends on LAI, LAE, LAD, photosynthesis rate/unit leaf area, LAR and ULR.

4. Grain yield: grain yield reflects the proportion of assimilates distribution between economic and total biomass. The length and number of spikelets, the weight of grains, the number of grains, cobs, pods, fruits e.t.c.

We use the formula as

\[ Y = Nr \times Ng \times Wg \]

Where \( Y = \text{grain yield} \), \( Nr = \text{the number of reproductive heads (head, ear, panicle)/unit ground area} \), \( Ng = \text{the number of grains/reproductive units} \) and \( Wg = \text{average weight per grain} \)

5. Dry matter production: profuse growth of roots absorbs high amount nutrients from soil and help in assimilation. Profuse branching and leaves also help in photosynthesis. High dry matter indicates high yield. The total yield of dry matter determines the close correlation with grain yield.
Pulse and oil seeds crop

Some modification based on the parts used as an economic part has to be considered in the discussion of physiological activities related to crop production are:

- Vegetative period
- Root to shoot ratio
- Grain size and test weight
- Plant population
- No. of pods or fruits/plant
- Grain and straw ratio
- Plant habit and growing season

Considering the above physiological factors which are directly reflected in the productivity by the physiological process such as:

- Photosynthesis
- Respiration
- Leaf canopy
- Source and sink distance
- Crop species growth analysis

➢ Growth analysis:
The following are the most important parameters in growth analysis:

Leaf Area Index (LAI)

\[
\text{LAI} = \frac{\text{leaf area}}{\text{soil area}} = \frac{LA}{A}; \text{ units } = \text{cm}^2/\text{soil}^2 \text{ or } \text{cm}^2/\text{soil}^2
\]

Measures the fraction of crop cover.

LAI is near 0 at planting, and is usually 2-3 at full canopy coverage.

The optimum leaf area index range for most crops is between 2.5 to 5.0 based on these facts.

1. The LAI during growth should be sufficient to intercept as much of the incoming radiation as possible.
2. The LAI should be of a magnitude which prevents parasitism: i.e. a condition of lower leaves using carbohydrates at a greater rate than they photosynthesize.
3. The LAI must suit the condition and purposes of which the crop is grown.

Leaf Area Duration (LAD) is used to describe the length of time the leaf area is functional e.g. a field corn might have LAI of 4.5 at the time of pollination, but it could be useful also to know how long this LAI is maintained. It has been established that the grain yield of cereals is related to LAI after the ears emerge but not before.

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**Optimum vs Critical Leaf Area**

**Optimum Leaf Area** is the leaf area at which the rate of dry matter production is max at a particular LAI and less at LAI below or above.

**Critical Leaf Area** is when the rate of dry matter production is constant after the maximum rate was reached. It is the LAI required to give 95% interception at noon.

**LAD:** The long term relationship of information found from the leaf Area Index, where the volume of ground covered in relation to upper leaf surface area is measured against time. It is the measure of its ability to produce leaf area on a unit land area throughout its life

\[
\text{LAD} = \frac{L_1 + L_2}{2} (t_x - t_{x-1}) \quad \text{or} \quad \frac{L_{x-1} + L_x}{2} (t_x - t_{x-1})
\]

Where \( L_1 \) is the leaf area in time \( t_1 \), \( L_2 \) is the leaf area in time \( t_2 \), \( L_x \) is the leaf area in time \( t_x \) and \( L_{x-1} \) is the leaf area in time \( t_{x-1} \)

**Leaf Area Ratio (LAR):** it is the ration between leaf area and total biomass accumulated in plant in a given point of time

\[\text{a) Over life of crop} \quad \text{LAR} = \frac{\text{final leaf area}}{\text{Final plant dry weight}} = \frac{\text{LA}}{\text{W}}\]

\[\text{b) Over any time Interval} \quad \text{LAR} = \frac{\text{leaf area}_2 - \text{leaf area}_1}{\text{plant dry weight}_2 - \text{plant dry weight}_1} = \frac{\text{LA}_2 - \text{LA}_1}{\text{W}_2 - \text{W}_1}\]

\( \text{; units = cm}^2 \text{ g}^{-1} \text{ or cm}^2/\text{g} \)

LAR is an indication of the efficiency of a given leaf area to produce a given plant size.

**Unit Leaf Rate:** it is the measure of increase in plant biomass/unit of assimilatory leaf area. Since leaf area changes with time, it is calculated as a mean value between two points in the life cycle of plant as:

\[
\text{ULR} = \frac{(W_2 - W_1)(\ln A_2 - \ln A_1)}{(A_2 - A_1)(t_2 - t_1)}
\]

Where \( W_2 \) is the plant weight from time \( t_1 \) and \( t_2 \), \( \ln A_1 \) and \( \ln A_2 \) logs of corresponding leaf area at harvest \( t_1 \) and \( t_2 \).

**Absolute Growth Rate (AGR)**

If you plot growth (size, mass or number) versus time, a constantly increasing growth curve is obtained. If you calculate the slope between any two times, you get the absolute growth rate, which is the change in actual growth over time. You get a different slope, hence different AGR

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for each pair of times chosen to calculate the slope. (Fig. 2.23A, Wareing and Philips 1981)

\[
AGR = \frac{(W_2 - W_1)}{(t_2 - t_1)}
\]

**RGR**

**Relative growth rate** (RGR) is a measure used in plant physiology to quantify the speed of plant growth. It is measured as the mass increase per aboveground biomass per day, for example as g g\(^{-1}\) d\(^{-1}\). It is considered to be the most widely used way of estimating plant growth, but has been criticised as calculations typically involve the destructive harvest of plants.

RGR is calculated using the following equation:

\[
RGR = \frac{(\ln W_2 - \ln W_1)}{(t_2 - t_1)}
\]

Where:

- \(\ln\) = natural logarithm
- \(t_1\) = time one (in days)
- \(t_2\) = time two (in days)
- \(W_1\) = Dry weight of plant at time one (in grams)
- \(W_2\) = Dry weight of plant at time two (in grams)

**Crop growth rate:**

It is the measure of increase in crop biomass per unit time.

\[
CGR = \frac{W_2 - W_1}{t_2 - t_1}
\]

Also, \(CGR = NAR \cdot LAI\); units = g cm\(^{-2}\)soil day\(^{-1}\) or g/cm\(^2\)soil/day

CGR measures the efficiency of production of a total field of plants over a given soil area.

**Net Assimilation Rate (NAR)**

\[
NAR = \frac{RGR}{LAR} = \frac{1}{LAR} \cdot RGR
\]

\[
= \frac{1}{LAR} \cdot \frac{\ln W_2 - \ln W_1}{t_2 - t_1}
\]

\[
= \frac{W_2 - W_1}{LAR_2 - LAR_1} \cdot \frac{\ln W_2 - \ln W_1}{t_2 - t_1};\; units = g\;cm^{-2}\;day^{-1}\; or\; g/cm^2/day
\]

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NAR measures the accumulation of plant dry weight per unit leaf area per unit time. It is a measure of efficiency of production.

- **Development analysis**
  - Leaf Production Rate: it can be estimated by counting the number of leaves on tagged plants at periodic intervals.
    \[ LPR = \frac{L_2 - L_1}{t_2 - t_1}, \]
    where the symbols have their usual meaning.

  - Panicle Emergence Rate: it is the rate at which the panicle emerges from the leaf sheath. As panicle emerges due to force for internodes elongation this parameter is important in moisture stress studies.
    \[ PER = \frac{PE_2 - PE_1}{t_2 - t_1}, \]
    \( PE \) is the length of panicle emerged in time \( t \).

  - Rate of Flowering: \( Fr_2 - Fr_1/t_2 - t_1 \) where \( Fr \) is the number of flower that appeared at time \( t \).

  - Days of flowering: it is number of days in which 50% of plants are flowered.

  - Days of maturity: it is the number of days in which plant attain maturity.

- **YIELD ANALYSIS**

  Harvest index = \[ \frac{\text{economic yield}}{\text{Biological yield}} \times 100 \]

  - Biological yield: it indicates the dry matter accumulation. It is dry matter produced per unit area.
  - Economic yield: also known as agricultural yield which is the total marketable yield produced per unit area.

  - Yield per unit area = \( \text{plants/unit area} \times (\text{heads/plant}) \times (\text{avg. seed/head}) \times (\text{mean wt./seed}) \)

  - Yield per unit area = \( \text{plants/unit area} \times (\text{n. of tillers with ears/plant}) \times (\text{mean no. of grains/ear}) \times (\text{mean grain weight}) \)

  - Yield capacity = \( (\text{no. of ears/m2}) \times (\text{no. of spikelets/ear}) \times (\text{potential size of a grain}) \)

Chlorophyll content in leaf is the indicator of biomass

Nutrient content (NPK, C:N) is also the indicator of nutrient present in plants.

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Chlorophyll estimation method: chlorophyll content gives a good idea about the productivity status of plant biomass. The relation of total chlorophyll to be photosynthetic rate is indicated by assimilation ratio of production /gm of chlorophyll.

**Growth analysis:** Many growth phenomena in nature exhibit a logarithmic or exponential increase. The size, mass or number increases by a constant, similar to simple compound interest. The principal (current size, mass or number) times the interest rate (growth rate) yields the interest (growth increase for that day). The interest is added to the principal, to yield a new principal. The new principal times the interest rate yields and even higher interest for the next day, which again is added back to the principal. So growth occurs at a compounded rate (logarithmic or exponential growth).

The growth in the base of a model---logistic curve which can be expressed by compound interest formula (Blackman, 1919)

\[ W_1 = W_0 e^{rt} \] ................................. (i)

Where, \( W \)--weight after growth, \( W_0 \)-- initial weight,  \( t \)-- time,  \( r \)--growth rate( rate of compound interest) and \( e \)--base of natural logarithm

The above equation can be restated in logarithm as

\[ \log e W_1 = \log e W_0 e^{rt} \]

Or, \( \log e W_1 = \log e W_0 + rt \log e^e \)

Or, \( \log e W_1 - \log e W_0 = rt \) (since \( \log e^e = 1 \))

i.e. \( \log e (W_1/W_0) = rt \)

or, \( r \) (efficiency index) = \( \frac{\log e W_1 - \log e W_0}{t} \)

- plant size also depends on the magnitude of growth rate
- the plant size with maximum efficiency index should perform better than that of low eff. index

Thank you very much for your cooperation…wishes you all the very best for the upcoming days…..

...........................................Subodh Khanal

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