BIO 208/BTG 310 – GENERAL PHYSIOLOGY

A SURVEY OF THE FUNDAMENTAL PRINCIPLES OF PLANT PHYSIOLOGY

Plant physiology is a sub-discipline 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, genetics, biophysics 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 parts of plant water relations, are studied by plant physiologists.

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. This includes study at many levels of scale of size and time. At the smallest scale are molecular interactions of photosynthesis and internal diffusion of water, minerals, and nutrients. At the largest scale are the processes of plant development, seasonality, dormancy, and reproductive control. 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.

PHOTOSYNTHESIS

Photosynthesis is a process used by plants and other organisms to convert light energy into chemical energy that can later be released to fuel the organisms' activities. This chemical energy is stored in carbohydrate molecules, such as sugars, which are synthesized from Carbon dioxide and water – hence the name *photosynthesis*, from the Greek φῶς, *phōs*, "light", and σύνθεσις, *synthesis*, "putting together". In most cases, oxygen is also released as a waste product. Most plants, most algae, and cyanobacteria perform photosynthesis; such organisms are called photoautotrophs. Photosynthesis is largely responsible for producing and maintaining the oxygen content of the Earth's atmosphere, and supplies all of the organic compounds and most of the energy necessary for life on Earth.

Photosynthesis (photo, light; synthesis, building up) consists in the building up of simple carbohydrates, such as sugars, in the green leaf by the chloroplasts in the presence of sunlight (as a source of energy) from Carbon dioxide and water absorbed from the air and the soil, respectively. The general consensus is that glucose is formed first and all other carbohydrates are derived from it. The process is accompanied by liberation of Oxygen. The volume of Oxygen liberated was found to be equal to the volume of CO_2 absorbed. It is noted that all the O_2 liberated in the process is released exclusively from H_2O and not from CO_2. Oxygen escapes from the plant body through the stomata.

The **formation of carbohydrates**, commonly called **carbon-assimilation**, is the monopoly of green plants, chlorophyll being indispensable to the process. By this process, not only are simple carbohydrates formed, but a considerable amount of energy (initially obtained from sunlight as radiant energy) is also transformed by the green cells into chemical energy and stored up as such in the organic substances formed. It must be noted that photosynthesis takes place only in the green cells and therefore, mainly in the leaf and to some extent in the green shoot. Under favourable conditions of light intensity and temperature, the rate of photosynthesis increases enormously and a tremendous amount of CO_2 is absorbed from the air for the process, so much so that on a windless day the CO_2 content of the air over a field crop may drop to 0.01% from the normal 0.03%.

MECHANISM OF PHOTOSYNTHESIS

Photosynthesis is the biological process by which some energy-rich Carbon-containing compounds are produced from CO_2 and H_2O by the illuminated green cells, liberating O_2 as a by-product. It is essentially an oxidation-reduction process by which Hydrogen is transferred from water to CO_2 through a 'carrier' substance. In the process, the volume of CO_2 absorbed is almost equal to that of O_2 liberated in most green plants (Boussingault, 1864). The overall reaction may be represented thus:

$$6CO_2 + 12H_2O \longrightarrow C_6H_{12}O_6 + 6H_2O + 6O_2$$

Photosynthesis involves two distinct phases, the first phase of **light reactions** and the second of **dark reactions**. Plenty of evidence, such as the **temperature co-efficient** obtained (**Q_{10}**) in experiments with intermittent light, the induction phase and the fact that O_2 liberation and CO_2 reduction are two completely separate processes, clearly indicate the existence of two phases in photosynthesis.

The **first phase of light reactions** comprises the conversion of radiant energy into chemical energy (located in phosphate bonds, i.e. **formation of ATP**), **photolysis (ionization) of water**, formation of a reducing agent (**$NADP.H_2$**) and **evolution of molecular O_2** (from water), which escapes to the atmosphere.

The **second phase of dark reactions** comprises the **reduction of CO_2 by $NADP.H_2$** and **the participation of ATP**, **a series of enzymes**, and **ribulose-di-phosphate** (CO_2 acceptor) to give rise to **hexose sugar** and regenerate ribulose-di-phosphate **(RuDP)**.

The **cycle of reactions through which the reduction of CO_2** (or CO_2 fixation) normally **takes place in the dark during photosynthesis** is called the **C_3 cycle** or Benson and Calvin cycle. However, there is an alternative cycle of reactions for CO_2 fixation in the dark during photosynthesis, known as the Hatch-Slack cycle, or **C_4 cycle**, which occurs in sugar-cane, maize, grasses etc. Light reactions take place in the lamellar region and dark reactions in the stroma region of chloroplasts (Park and Pou, 1961).

Light reactions: These reactions **take place in the green cells of plants** in the presence of light. **Chlorophyll absorbs light energy**, which is then **converted into chemical energy** and **temporarily stored** in **two compounds**, namely, Adenosine-tri-phosphate (**ATP**) and reduced Nicotinamide adenine dinucleotide phosphate (**$NADP.H_2$**). **There are two ways in which ATP is formed, but $NADP.H_2$ is formed only in one way**.

The photosynthesis (**ionization**) **of water also takes place in this phase**. **Water supplies Hydrogen ions for the reduction of oxidized NADP into NADP**.H_2 and is the source of Oxygen which is evolved during photosynthesis. When light falls upon a chlorophyll molecule, it becomes excited. This means when a **photon** (a quantum of light, which is a discrete packet of light energy) strikes the chlorophyll molecule, an electron is lifted into a new orbit, or a higher energy level, **helping the chlorophyll molecule to gain an amount of energy equivalent to that of a photon**.

It is now known that the **main light-absorbing pigment is Chlorophyll-a**. The minimum number of Chlorophyll-a molecules and accessory pigments required to absorb and convert a photon or quantum of light constitute a unit, called **photosynthetic unit**. Though, many chlorophyll-a molecules are grouped together in such unit, it is **only one chlorophyll-a molecule** that **acts as the reaction centre** or **trapping centre** and can donate an electron. This chlorophyll-a molecule is termed **P_{700}**. The **light absorbed** by the **remaining chlorophyll-a molecule passes from one molecule to another, and finally transferred to P_{700} chlorophyll-a molecule**. These energy transferring chlorophyll-a molecules are called **antenna chlorophyll molecules**.

The electron (brought to a higher energy level) expelled by P_{700} is picked up by an electron carrier called **Ferredoxin** (an iron-containing co-enzyme present in the chloroplast; the iron gets oxidized by donating an electron). From Ferredoxin, the electron passes through a series of other electron carriers, such as cytochrome, quinones (plastoquinones) and vitamin K) etc. **During the transfer of the electron from one carrier to another, the high energy of the electron is utilized for the addition of phosphate radical to ADP by the phosphate bond to form ATP** (two or more ATP molecules are formed in this cycle). Finally, the excited electron gets back to its ground state and is returned to the chlorophyll. The chlorophyll, therefore, gets back the same electron. This is a closed circuit flow of electrons and is known as **cyclic photophosphorylation**.

The second way that ATP is formed during photosynthesis is called **non cyclic photophosphorylation**.

Dark Reactions: These reactions, which constitute the second phase of photosynthesis, are not dependent on light and chlorophyll. This process utilizes the potential chemical energy stored in green cells during light reactions. The first step of dark reactions is Carbon dioxide fixation, in which RuDP functions as Carbon dioxide acceptor. CO_2 is then reduced by **$NADP.H_2$**. Working on the unicellular algae *Chlorella*, Benson and Calvin (1950) showed with the help of radioactive Carbon^{14}, that Phosphoglyceric acid is formed as an intermediate product during the process. Phosphoglyceric acid gets converted to Phosphoglyceraldehyde. For every six molecules of CO_2 absorbed and reduced, 12 molecules of Phosphoglyceraldehyde are formed, two of which are utilized to give rise to one molecule of sugar, and 10 to regenerate RuDP molecules. This process is called the C_3-Cycle or Calvin Cycle. It is also known that the reactions leading to the formation of glucose are essentially a reversal of the glycolytic phase of cellular respiration and that they take place under the action of specific enzymes.

In recent years, the use of radioactive isotopes and chromatography has helped the study of dark reactions. It has been suggested that 5-Carbon ribulose phosphate is activated by ATP to form ribulose diphosphate (also a 5-carbon compound). The latter combines with CO_2 and is carboxylated to an unknown 6-carbon compound. This unknown compound, along with water molecules, splits into two parts – one part forms 3-Carbon phosphoglyceric acid and the other part may enter the mitochondria and follow the path of cellular respiration. The two molecules of phosphoglyceric acid, formed per molecule of CO_2, are reduced to two molecules of 3-Phosphoglyceraldehyde by the reducing agent NADP.H_2, in the presence of ATP, both formed during light reactions. 3-Phosphoglyceraldehyde plays a key role in further transformations.

Some molecules of 3-Phosphoglyceraldehyde isomerise to form dihydroxyacetone phosphate. Then, some dihydroxyacetone phosphate molecules combine with some 3-Phosphoglyceraldehyde molecules to form Fructose 1-6-diphosphate molecules. These lose one phosphate each and convert to Fructose-6-phosphate molecules. Fructose molecules undergo reorganization to become glucose (sugar).

ROLES PLAYED BY LIGHT AND CHLOROPHYLL

LIGHT is an essential condition for initiating the process of photosynthesis. The part played by it in any photochemical reaction is not, however, completely understood, and consequently, a clear and complete explanation of the action of light in photosynthesis cannot be given. Enough (suffice) to say that the light energy absorbed by chlorophyll is effective in splitting water molecules at the initial stage of photosynthesis (**photolysis of water**) and in this process, **it becomes transformed into chemical energy** for further action i.e. **reduction and fixation of CO_2 in dark reactions**. It has been estimated that an average green leaf absorbs about 80-85% light. In photosynthesis, however, only about 1% light is utilized. A portion of light may be reflected from the leaf-surface and from the chloroplasts; a portion is used in transpiration. Certain experiments have proved that only certain rays (not all) of light are used for photosynthesis.

CHLOROPHYLL is indispensable for photosynthesis. However, its exact role in the process is not known except that (a) it absorbs light energy from sunlight and becomes 'activated' or 'excited', i.e. its energy level increases above normal to initiate the process of photosynthesis,

(b) the extra energy stored in chlorophyll now goes to break up water molecules at the initial stage of photosynthesis, (c) a part of this energy goes to form a reducing agent, TPN in all probability, which accepts Hydrogen from water for reduction of CO_2, (d) another part of this energy goes to synthesize ATP, and (e) chlorophyll acts as a catalytic agent. The amount of chlorophyll or the constituent pigments remain unaltered through even a prolonged period of photosynthesis.

Photolysis of Water: The initial and essential part of photosynthesis lies in photolysis i.e. splitting of a water molecule into its two components. This is essentially a light reaction taking place in the body of the chloroplast. In fact, all photosynthetic reactions, as shown by Arnon *et al*., 1954 occur in the grana of the chloroplast. In this respect, the chloroplast may be regarded as a complete unit. Under the influence of light energy and the catalytic action of chlorophyll, water, a substance of low energy value, is split up into Oxygen (O_2) and Hydrogen (2H). Oxygen escapes to the atmosphere, while Hydrogen atoms (or their electrons) combine with a reducing agent, most likely NADP formed in the chloroplast. NADP becomes reduced to $TPNH_2$, a substance of high energy value. This further result in the synthesis of ATP from ADP. It is possibly during these reactions that light energy is converted to chemical energy which is needed for further reactions that immediately follow. **It should be specially noted that all the Oxygen evolved in photosynthesis is released from water**. Further, **the volume of the O_2 released in photosynthesis has been found to be equal to the volume of the CO_2 absorbed**.

$$6CO_2 + 12H_2O \xrightarrow[\text{chlorophyll}]{\text{light energy}} C_6H_{12}O_6 + 6H_2O + 6O_2$$

RESPIRATION

Respiration is essentially a process of oxidation and decomposition of organic compounds, particularly simple carbohydrates such as glucose, in the living cells with the release of energy. The most important feature of respiration is that by this oxidative process, the potential energy stored in the organic compounds in living cells is released in a step-wise manner, in the form of active or kinetic energy, under the influence of a series of enzymes and is made available, at least partly, to the protoplasm for its manifold vital activities. Often, a considerable amount of energy

escapes from the plant body in the form of heat, as in germinating seeds. The reserve food materials that undergo oxidation are mostly simple carbohydrates, principally glucose, and sometimes also, particularly in the absence of glucose, other substances such as complex carbohydrates, proteins and fats. These are first hydrolysed and then oxidized. The main facts associated with respiration are: (1) Consumption of atmospheric O_2. (2) Oxidation and decomposition of a portion of the stored food, resulting in a loss of dry weight, as seen in seeds germination in the dark. (3) Liberation of CO_2 and a small quantity of water (the volume of CO_2 liberated being equal to the volume of O_2 consumed). (4) Most important, release of energy by the breakdown of organic food. The overall chemical reaction may be represented thus:
$C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O$ + Energy (ATP) (Sugar + Oxygen = Carbondioxide + water + Energy). This shows that for oxidation of one molecule of sugar, six molecules of Oxygen are used and that six molecules of each CO_2 and H_2O are also formed. By burning sugar at a high temperature, CO_2 and H_2O are also formed, but in living cells, this process is carried out by a series of enzymes at a comparatively low temperature. Oxidation may be complete, as shown in the formula, with the formation of Carbondioxide and water as end-products, the former escaping from the plant body and the latter getting mixed up with the general mass of water in the cells. Otherwise, it may be incomplete with the formation of some organic acid or Ethyl alcohol and CO_2, as shown by the equation: **$C_6H_{12}O_6 = 2C_2H_5OH + CO_2$** (Sugar = Ethyl alcohol + CO_2). In respiration of plants, the oxygen gas, after entering through the stomata and lenticels, diffuses through the intercellular spaces into the living cells and slowly oxidizes not only glucose and other carbohydrates, but also (though less frequently) other organic materials e.g. fats, proteins, organic acids and even protoplasm under extreme conditions. The CO_2 that is formed in respiration diffuses through the intercellular spaces and finally escapes through the stomata and the lenticels into the surrounding air. A portion of it may be retained in the cells and used for photosynthesis. In submerged aquatic plants, the surrounding water containing dissolved air supplies the gases required for respiration and carbon-assimilation.
All the living cells of a plant, however deeply seated they may be, must respire day and night in order to live. If the supply of air to a plant is cut off (by growing it in an atmosphere devoid of Oxygen), it will soon die. The growing organs like the floral and vegetative buds; germinating seeds; stem and root tips, respire actively, while the adult organs do so (i.e. respire) comparatively slowly. Gases normally enter the plant through the **stomata**, but these are closed

at night. So, to facilitate the interchange of gases the concerned, special structures are developed on the branches. These are the **lenticels** which unlike the stomata; remain open. **For easy diffusion of gases in the interior of the plant body, plants have developed a network of air-cavities and intercellular spaces** which are connected throughout and are continuous with the stomata and the lenticels.

In **respiration**, the cells are continually **exhaling CO_2** and in **photosynthesis**, **the green cells are continually making use of this gas during the daytime and giving off O_2**. But the latter process (photosynthesis) goes on more vigorously than the former (respiration) and practically masks it. Thus, in the green parts of plants, the composition of the intercellular air varies, becoming **richer in O_2 during the daytime**; and **richer in CO_2 at night**. The amount of Nitrogen varies very little, as this gas is not made use of by the protoplasm. Since plants are continually exhaling CO_2 in respiration, the atmosphere has a tendency to become richer in this gas, especially at night. CO_2 is a suffocating gas and tends to vitiate the atmosphere; but then during the day, the green plants absorb it for photosynthesis and give out O_2 which goes back to the atmosphere. The atmosphere is, thus, purified and the composition of the air remains constant.

AEROBIC AND ANAEROBIC RESPIRATION (aer: air; an: not; bios: life)

Normally, free Oxygen is used in respiration, resulting in complete oxidation of stored food and formation of CO_2 and H_2O as end-products, this is known as aerobic respiration. A considerable amount of energy is released by this process, as represented thus:

$C_6H_{12}O_6 + 6O_2 = 6CO_2 + 6H_2O + 674$ Kilo-cal (sugar + oxygen = carbondoixide + water + 674 Kilo-cal. of energy).

Under **certain conditions**, as in the **absence** of **free O_2**, many tissues of higher plants, seeds in storage, fleshy fruits and succulent plants like ‘cacti’ temporarily take to a kind of respiration called **anaerobic respiration**. Such respiration results in incomplete oxidation of stored food and formation of CO_2 and Ethyl alcohol, and sometimes also various organic acids e.g. Malic, Citric, Oxalic, Tartaric etc. The energy released by this process is not sufficient to maintain the activity of the protoplasm. It may be represented by the equation:

$C_6H_{12}O_6 = 2C_2H_5OH + 2CO_2 + 28$ Kilocalorie (Sugar = Ethyl alcohol + Carbondioxide + 28 Kilo-calorie of energy).

It is otherwise known as **intramolecular respiration** because in this process, intramolecular oxidation of sugar and other compounds takes place without the use of free O_2. Anaerobic respiration may continue for only a limited period of time, at most a few days, after which death ensues, evidently due to low production of energy and accumulation of toxic substances in the cells. In certain microorganisms (certain bacteria, yeast and some other fungi) however, the fundamental process of energy-release is anaerobic respiration. Anaerobic respiration resulting in the production of alcohol is otherwise called alcoholic fermentation.

MECHANISM OF RESPIRATION

Chemical changes in respiration (from the breakdown of glucose to the release of CO_2 and H_2O) are more or less definitely known. It is known that the whole process is controlled by a group of different kinds of complex enzymes, the respiratory enzymes, which work step by step. We also know that it is complete in two distinct phases: an anaerobic phase and an aerobic phase. **The first phase, which is incomplete oxidation of glucose to Pyruvic acid through a chain of intermediate reactions, is called glycolysis**. This phase takes place in the absence of O_2, i.e. it does not require O_2. **The second phase, which involves complete oxidation of the Pyruvic acid to CO_2 and H_2O, is called Krebs cycle; and it takes place in the presence of O_2**. Several reactions occur in the whole process, each reaction being controlled by a specific enzyme.

Pathway of Glycolysis (Anaerobic oxidation of Pyruvic acid)

Krebs or Citric acid cycle (Aerobic oxidation of Pyruvic acid to CO_2 and H_2O)

CELL-WATER RELATIONSHIP

A cell is the simplest unit of life; the basic structural unit of an organism. A cell can also be defined as the basic structure and function of all life. Plant organization varies from single cells to aggregation of cells to complex multicellular structures. With increasing complexity, there are increasingly sophisticated systems for absorbing water, moving it to large distances and conserving it but fundamentally, the cell remains the central unit that controls the plant response to water. The driving forces for water movement are generated in the cells, and growth and metabolism occur in the aqueous medium provided by the cells. The cell properties can change and result in acclimation to the water environment. As a consequence, many features of complex multicellular plants can be understood only from knowledge of the cell properties.

STRUCTURE: The plant cell consists of a multicompartmented cytoplasm bounded on the outside by a membrane and cell wall. There usually is a dilute solution on the outside, but in some instances, there may be a moderately concentrated solution as in seawater or around embryonic cells. On the inside, there always is a concentrated solution that contains metabolites, inorganic salts and macromolecules in varying concentrations depending on the location. The membrane bounding the outside of the cytoplasm is the plasmalemma which is highly permeable to water but only slowly but selectively permeable to solutes. The cell wall outside the plasmalemma is porous and permits water and solutes of low molecular weight to move rapidly to and from the plasmalemma. Inside the cytoplasm are compartments or organelles e.g. vacuoles, nucleus, mitochondria and plastids, each bounded by a membrane similar to the plasmalemma and capable of exchanging water and solutes with the surrounding cytosol. Each organelle contains its own unique composition of solute. The plasmalemma is thus the primary barrier controlling the molecular traffic in and out of the cell but the cell wall and internal membranes also play a role.

The high concentration of solute inside the cell dilutes the internal water compared to that outside and water enters in response, causing the cell to swell. The plasmalemma has insufficient strength to resist the swelling but it is supported by the structurally tough and often rigid wall which resists enlargement. As a result, the swelling causes the wall to stretch and becomes turgid building up turgor pressure inside the cell. Without the wall, the plasmalemma will rupture but with the support of the wall, the plasmalemma is pressed tightly against the wall microstructure. The wall sometimes can stretch by a considerable amount, and the membrane inside must be capable of stretching as well. The resistance to stretch gives structural rigidity that contributes to the form and strength of tissues. Much of the form of leaves and stems of herbaceous plants results from the turgor pressure developing in their cells. Cells loose water when solute concentrations are high outside, or when evaporation occurs, and they shrink as the volume of water decreases inside. The membranes cannot resist the shrinkage, and the organelles become distorted when dehydration is severe. The cell wall often develops folds as the cell shrinks and the folding deforms the adjacent plasmalemma. In some cells, the walls are stiffened by the deposition of layers of rigid wall materials and folding does not occur. In such cells, the walls resist shrinkage and the cell contents may come under tension.