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SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF BIOTECHNOLOGY

UNIT – I– PLANT BIOCHEMISTRY– SBC3201

Unit 1

Plant Physiology

The description of structure and variation of living organisms over a period of time, ended up as two, apparently irreconcilable perspectives on biology. The two perspectives essentially rested on two levels of organisation of life forms and phenomena. One described at organismic and above level of organisation while the second described at cellular and molecular level of organisation. The first resulted in ecology and related disciplines. The second resulted in physiology and biochemistry. Description of physiological processes, in flowering plants as an example, is what is given in the chapters in this unit. The processes of mineral nutrition of plants, photosynthesis, transport, respiration and ultimately plant growth and development are described in molecular terms but in the context of cellular activities and even at organism level. Wherever appropriate, the relation of the physiological processes to environment is also discussed.

MELVIN CALVIN born in Minnesota in April, 1911, received his Ph.D. in Chemistry from the University of Minnesota. He served as Professor of Chemistry at the University of California, Berkeley. Just after world war II, when the world was under shock after the Hiroshima-Nagasaki bombings, and seeing the illeffects of radio-activity, Calvin and co-workers put radioactivity to beneficial use. He along with J.A. Bassham studied reactions in green plants forming sugar and other substances from raw materials like carbon dioxide, water and minerals by labelling the carbon dioxide with C14. Calvin proposed that plants change light energy to chemical energy by transferring an electron in an organised array of pigment molecules and other substances. The mapping of the pathway of carbon assimilation in photosynthesis earned him Nobel Prize in 1961. The principles of photosynthesis as established by Calvin are, at present, being used in studies on renewable resource for energy and materials and basic studies in solar energy research.

TRANSPORT IN PLANTS

Have you ever wondered how water reaches the top of tall trees, or for that matter how and why substances move from one cell to the other, whether all substances move in a similar way, in the same direction and whether metabolic energy is required for moving substances. Plants need to move molecules over very long distances, much more than animals do; they also do not have a circulatory system in place. Water taken up by the roots has to reach all parts of the plant, up to the very tip of the growing stem. The photosynthates or food synthesised by the leaves have also to be moved to all parts including the root tips embedded deep inside the soil. Movement across short distances, say

within the cell, across the membranes and from cell to cell within the tissue has also to take place. To understand some of the transport processes that take place in plants, one would have to recollect one's basic knowledge about the structure of the cell and the anatomy of the plant body. We also need to revisit our understanding of diffusion, besides gaining some knowledge about chemical potential and ions. When we talk of the movement of substances we need first to define what kind of movement we are talking about, and also what substances we are looking at. In a flowering plant the substances that would need to be transported are water, mineral nutrients, organic nutrients and plant growth regulators. Over small distances substances move by diffusion and by cytoplasmic streaming supplemented by active transport. Transport over longer distances proceeds through the vascular system (the xylem and the phloem) and is called translocation. An important aspect that needs to be considered is the direction of transport. In rooted plants, transport in xylem (of water and minerals) is essentially unidirectional, from roots to the stems. Organic and mineral nutrients however, undergo multidirectional transport. Organic compounds synthesised in the photosynthetic leaves are exported to all other parts of the plant including storage organs. From the storage organs they are later re-exported. The mineral nutrients are taken up by the roots and transported upwards into the stem, leaves and the growing regions. When any plant part undergoes senescence, nutrients may be withdrawn from such regions and moved to the growing parts. Hormones or plant growth regulators and other chemical signals are also transported, though in very small amounts, sometimes in a strictly polarised or unidirectional manner from where they are synthesised to other parts. Hence, in a flowering plant there is a complex traffic of compounds (but probably very orderly) moving in different directions, each organ receiving some substances and giving out some others.

1.1 MEANS OF TRANSPORT

1.1.1 Diffusion Movement by diffusion is passive, and may be from one part of the cell to the other, or from cell to cell, or over short distances, say, from the intercellular spaces of the leaf to the outside. No energy expenditure takes place. In diffusion, molecules move in a random fashion, the net result being substances moving from regions of higher concentration to regions of lower concentration. Diffusion is a slow process and is not dependent on a 'living system'. Diffusion is obvious in gases and liquids, but diffusion in solids is more likely rather than of solids. Diffusion is very important to plants since it is the only means for gaseous movement within the plant body. Diffusion rates are affected by the gradient of concentration, the permeability of the membrane separating them, temperature and pressure.

1.1.2 Facilitated Diffusion As pointed out earlier, a gradient must already be present for diffusion to occur. The diffusion rate depends on the size of the substances; obviously smaller substances diffuse

faster. The diffusion of any substance across a membrane also depends on its solubility in lipids, the major constituent of the membrane. Substances soluble in lipids diffuse through the membrane faster. Substances that have a hydrophilic moiety, find it difficult to pass through the membrane; their movement has to be facilitated. Membrane proteins provide sites at which such molecules cross the membrane. They do not set up a concentration gradient: a concentration gradient must already be present for molecules to diffuse even if facilitated by the proteins. This process is called facilitated diffusion. In facilitated diffusion special proteins help move substances across membranes without expenditure of ATP energy. Facilitated diffusion cannot cause net transport of molecules from a low to a high concentration – this would require input of energy. Transport rate reaches a maximum when all of the protein transporters are being used (saturation).

Facilitated diffusion is very specific: it allows cell to select substances for uptake. It is sensitive to inhibitors which react with protein side chains. The proteins form channels in the membrane for molecules to pass through. Some channels are always open; others can be controlled. Some are large, allowing a variety of molecules to cross. The porins are proteins that form large pores in the outer membranes of the plastids, mitochondria and some bacteria allowing molecules up to the size of small proteins to pass through. Figure 1.1 shows an extracellular molecule bound to the transport protein; the transport protein then rotates and releases the molecule inside the cell, e.g., water channels – made up of eight different types of aquaporins.

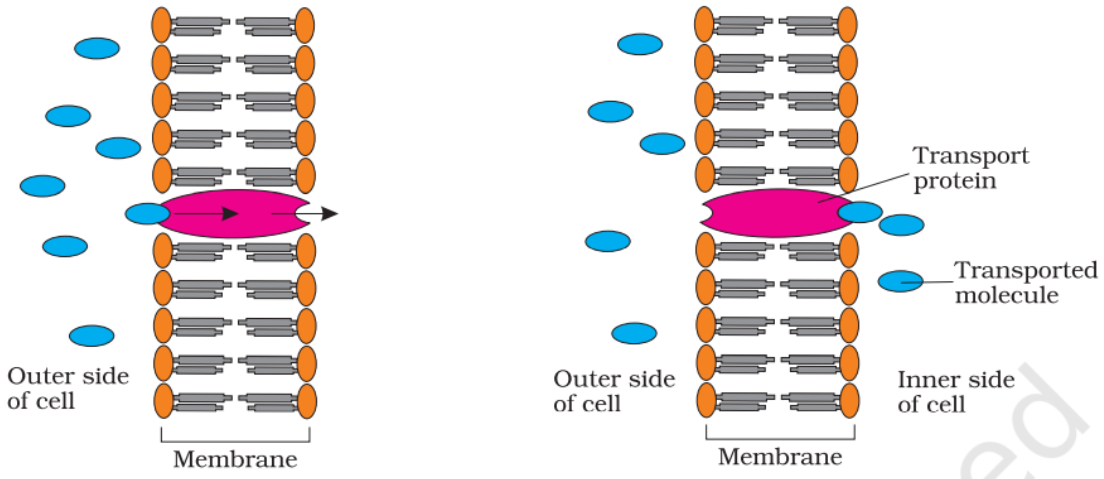

Fig 1.1 facilitated diffusion

1.1.2.1 Passive symports and antiports Some carrier or transport proteins allow diffusion only if two types of molecules move together. In a symport, both molecules cross the membrane in the same direction; in an antiport, they move in opposite directions (Figure 1.2). When a molecule moves across a membrane independent of other molecules, the process is called uniport.

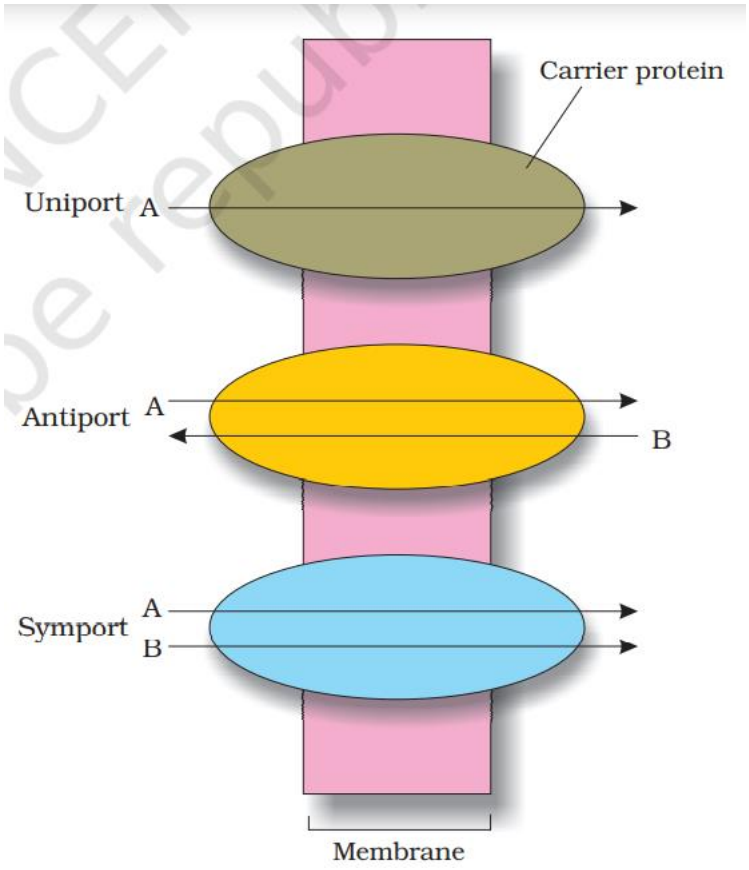

Fig 1.2. facilitated diffusion

1.1.3 Active Transport Active transport uses energy to transport and pump molecules against a concentration gradient. Active transport is carried out by specific membrane-proteins. Hence different proteins in the membrane play a major role in both active as well as passive transport. Pumps are proteins that use energy to carry substances across the cell membrane. These pumps can transport substances from a low concentration to a high concentration ('uphill' transport). Transport rate reaches a maximum when all the protein transporters are being used or are saturated. Like enzymes the carrier protein is very specific in what it carries across the membrane. These proteins are sensitive to inhibitors that react with protein side chains.

1.1.4 Comparison of Different Transport Processes Table 1.1 gives a comparison of the different transport mechanisms. Proteins in the membrane are responsible for facilitated diffusion and active transport and hence show common characterstics of being highly selective; they are liable to saturate, respond to inhibitors and are under hormonal regulation. But diffusion whether facilitated or not – take place only along a gradient and do not use energy.

TABLE 1.1 Comparison of Different Transport Mechanisms

Property	Simple Diffusion	Facilitated Transport	Active Transport
Requires special membrane proteins	No	Yes	Yes
Highly selective	No	Yes	Yes
Transport saturates	No	Yes	Yes
Uphill transport	No	No	Yes
Requires ATP energy	No	No	Yes

1.2 PLANT-WATER RELATIONS

Water is essential for all physiological activities of the plant and plays a very important role in all living organisms. It provides the medium in which most substances are dissolved. The protoplasm of the cells is nothing but water in which different molecules are dissolved and (several particles) suspended. A watermelon has over 92 per cent water; most herbaceous plants have only about 10 to 15 per cent of its fresh weight as dry matter. Of course, distribution of water within a plant varies – woody parts have relatively very little water, while soft parts mostly contain water. A seed may appear dry but it still has water – otherwise it would not be alive and respiring! Terrestrial plants take up huge amount water daily but most of it is lost to the air through evaporation from the leaves, i.e., transpiration. A mature corn plant absorbs almost three litres of water in a day, while a mustard plant absorbs water equal to its own weight in about 5 hours. Because of this high demand for water, it is not surprising that water is often the limiting factor for plant growth and productivity in both agricultural and natural environments.

1.2.1 Water Potential

To comprehend plant-water relations, an understanding of certain standard terms is necessary. Water potential (Ψw) is a concept fundamental to understanding water movement. Solute potential (Ψs) and pressure potential (Ψp) are the two main components that determine water potential. Water molecules possess kinetic energy. In liquid and gaseous form they are in random motion that is both rapid and constant. The greater the concentration of water in a system, the greater is its kinetic energy or 'water potential'. Hence, it is obvious that pure water will have the greatest water potential. If two systems containing water are in contact, random movement of water molecules will result in net movement of water molecules from the system with higher energy to the one with lower energy. Thus water will move from the system containing water at higher water potentialto the one having low water potential . This process of movement of substances down a gradient of free energy is called diffusion. Water potential is denoted by the Greek symbol Psi or Ψ and is expressed in pressure units such as pascals (Pa). By convention, the water potential of pure water at standard temperatures,

which is not under any pressure, is taken to be zero. If some solute is dissolved in pure water, the solution has fewer free water molecules and the concentration (free energy) of water decreases, reducing its water potential. Hence, all solutions have a lower water potential than pure water; the magnitude of this lowering due to dissolution of a solute is called solute potential or Ψs . Ψs is always negative. The more the solute molecules, the lower (more negative) is the Ψs .For a solution at atmospheric pressure (water potential) Ψw = (solute potential) Ψs . If a pressure greater than atmospheric pressure is applied to pure water or a solution, its water potential increases. It is equivalent to pumping water from one place to another. Can you think of any system in our body where pressure is built up? Pressure can build up in a plant system when water enters a plant cell due to diffusion causing a pressure built up against the cell wall, it makes the cell turgid (see section 1.2.2); this increases the pressure potential. Pressure potential is usually positive, though in plants negative potential or tension in the water column in the xylem plays a major role in water transport up a stem. Pressure potential is denoted as Ψp . Water potential of a cell is affected by both solute and pressure potential.

The relationship between them is as follows: $\Psi w = \Psi s + \Psi p$

1.2.2 Osmosis

The plant cell is surrounded by a cell membrane and a cell wall. The cell wall is freely permeable to water and substances in solution hence is not a barrier to movement. In plants the cells usually contain a large central vacuole, whose contents, the vacuolar sap, contribute to the solute potential of the cell. In plant cells, the cell membrane and the membrane of the vacuole, the tonoplast together are important determinants of movement of molecules in or out of the cell. Osmosis is the term used to refer specifically to the diffusion of water across a differentially- or selectively permeable membrane. Osmosis occurs spontaneously in response to a driving force. The net direction and rate of osmosis depends on both the pressure gradient and concentration gradient. Water will move from its region of higher chemical potential (or concentration) to its region of lower chemical potential until equilibrium is reached. At equilibrium the two chambers should have nearly the same water potential. You may have made a potato osmometer in your earlier classes in school. If the potato tuber is placed in water, the water enters the cavity in the potato tuber containing a concentrated solution of sugar due to osmosis.

Study Figure 1.3 in which the two chambers, A and B, containing solutions are separated by a semi-permeable membrane.

(a) Solution of which chamber has a lower water potential?

(b) Solution of which chamber has a lower solute potential?

(c) In which direction will osmosis occur?

(d) Which solution has a higher solute potential?

(e) At equilibrium which chamber will have lower water potential?

(f) If one chamber has a Ψ of – 2000 kPa, and the other – 1000 kPa, which is the chamber that has the higher Ψ?

(g) What will be the direction of the movement of water when two solutions with Ψw = 0.2 MPa and Ψw = 0.1 MPa are separated by a selectively permeable membrane?

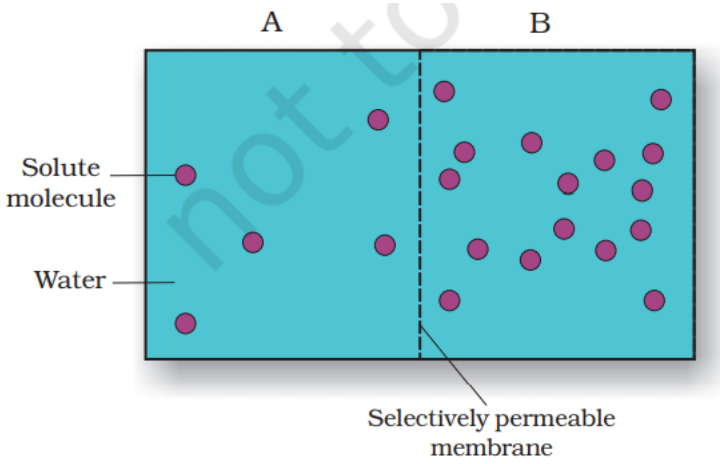

Figure 1.3

Let us discuss another experiment where a solution of sucrose in water taken in a funnel is separated from pure water in a beaker by a selectively permeable membrane (Figure 1.4). You can get this kind of a membrane in an egg. Remove the yolk and albumin through a small hole at one end of the egg, and place the shell in dilute solution of hydrochloric acid for a few hours. The egg shell dissolves leaving the membrane intact. Water will move into the funnel, resulting in rise in the level of the solution in the funnel. This will continue till the equilibrium is reached. In case sucrose does diffuse out through the membrane, will this equilibrium be ever reached? External pressure can be applied from the upper part of the funnel such that no water diffuses into the funnel through the membrane. This pressure required to prevent water from diffusing is in fact, the osmotic pressure and this is the function of the solute concentration; more the solute concentration, greater will be the pressure required to prevent water from diffusing in. Numerically osmotic pressure is equivalent to the osmotic potential, but the sign is opposite. Osmotic pressure is the positive pressure applied, while osmotic potential is negative.

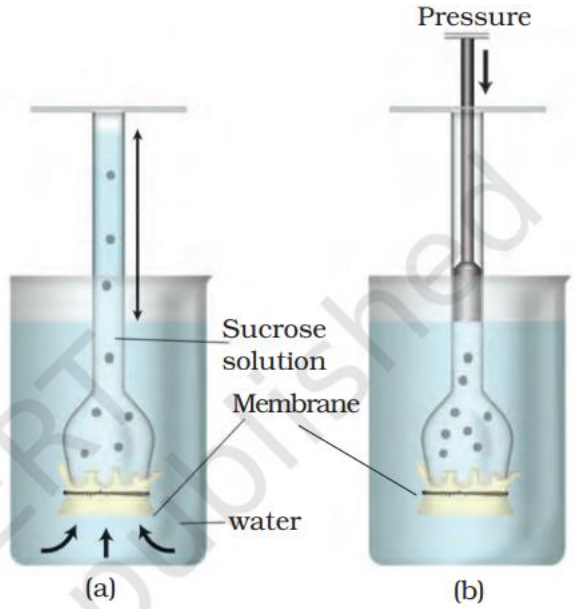

Figure .1.4 A demonstration of osmosis. A thistle funnel is filled with sucrose solution and kept inverted in a beaker containing water. (a) Water will diffuse across the membrane (as shown by arrows) to raise the level of the solution in the funnel (b) Pressure can be applied as shown to stop the water movement into the funnel

1.2.3 Plasmolysis The behaviour of the plant cells (or tissues) with regard to water movement depends on the surrounding solution. If the external solution balances the osmotic pressure of the cytoplasm, it is said to be isotonic. If the external solution is more dilute than the cytoplasm, it is hypotonic and if the external solution is more concentrated, it is hypertonic. Cells swell in hypotonic solutions and shrink in hypertonic ones.

Plasmolysis occurs when water moves out of the cell and the cell membrane of a plant cell shrinks away from its cell wall. This occurs when the cell (or tissue) is placed in a solution that is hypertonic (has more solutes) to the protoplasm. Water moves out; it is first lost from the cytoplasm and then from the vacuole. The water when drawn out of the cell through diffusion into the extracellular (outside cell) fluid causes the protoplast to shrink away from the walls. The cell is said to be plasmolysed. The movement of water occurred across the membrane moving from an area of high water potential (i.e., the cell) to an area of lower water potential outside the cell (Figure 1.5). What occupies the space between the cell wall and the shrunken protoplast in the plasmolysed cell? When the cell (or tissue) is placed in an isotonic solution, there is no net flow of water towards the inside or

outside. If the external solution balances the osmotic pressure of the cytoplasm it is said to be isotonic. When water flows into the cell and out of the cell and are in equilibrium, the cells are said to be flaccid. The process of plasmolysis is usually reversible. When the cells are placed in a hypotonic solution (higher water potential or dilute solution as compared to the cytoplasm), water diffuses into the cell causing the cytoplasm to build up a pressure against the wall, that is called turgor pressure. The pressure exerted by the protoplasts due to entry of water against the rigid walls is called pressure potential Ψp. . Because of the rigidity of the cell wall, the cell does not rupture. This turgor pressure is ultimately responsible for enlargement and extension growth of cells. What would be the Ψp of a flaccid cell? Which organisms other than plants possess cell wall ?

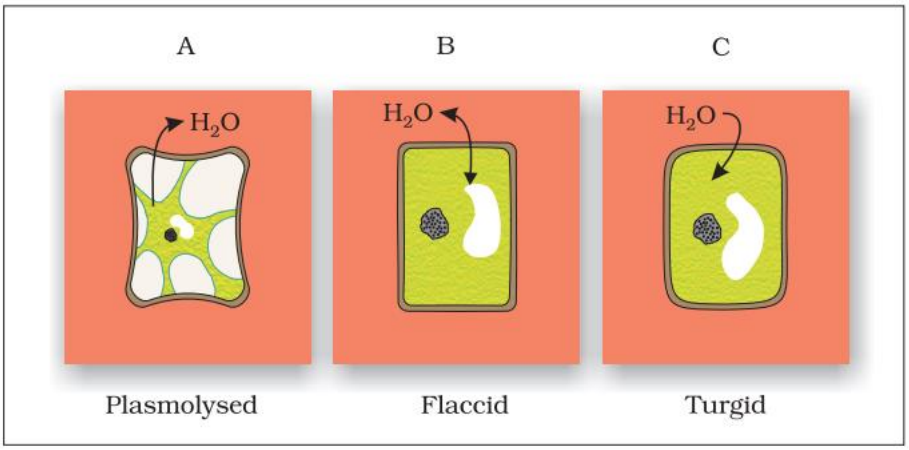

Figure 1.5 Plant cell plasmolysis

1.2.4 Imbibition

Imbibition is a special type of diffusion when water is absorbed by solids – colloids – causing them to increase in volume. The classical examples of imbibition are absorption of water by seeds and dry wood. The pressure that is produced by the swelling of wood had been used by prehistoric man to split rocks and boulders. If it were not for the pressure due to imbibition, seedlings would not have been able to emerge out of the soil into the open; they probably would not have been able to establish! Imbibition is also diffusion since water movement is along a concentration gradient; the seeds and other such materials have almost no water hence they absorb water easily. Water potential gradient between the absorbent and the liquid imbibed is essential for imbibition. In addition, for any substance to imbibe any liquid, affinity between the adsorbant and the liquid is also a pre-requisite.

1.3 LONG DISTANCE TRANSPORT OF WATER

At some earlier stage you might have carried out an experiment where you had placed a twig bearing white flowers in coloured water and had watched it turn colour. On examining the cut end of the twig after a few hours you had noted the region through which the coloured water moved. That experiment very easily demonstrates that the path of water movement is through the vascular bundles, more specifically, the xylem. Now we have to go further and try and understand the mechanism of movement of water and other substances up a plant. Long distance transport of substances within a plant cannot be by diffusion alone. Diffusion is a slow process. It can account for only short distance movement of molecules. For example, the movement of a molecule across a typical plant cell (about 50 µm) takes approximately 2.5 s. At this rate, can you calculate how many years it would take for the movement of molecules over a distance of 1 m within a plant by diffusion alone? In large and complex organisms, often substances have to be moved to long distances. Sometimes the sites of production or absorption and sites of storage are too far from each other; diffusion or active transport would not suffice. Special long distance transport systems become necessary so as to move substances across long distances and at a much faster rate. Water and minerals, and food are generally moved by a mass or bulk flow system. Mass flow is the movement of substances in bulk or en masse from one point to another as a result of pressure differences between the two points. It is a characteristic of mass flow that substances, whether in solution or in suspension, are swept along at the same pace, as in a flowing river. This is unlike diffusion where different substances move independently depending on their concentration gradients. Bulk flow can be achieved either through a positive hydrostatic pressure gradient (e.g., a garden hose) or a negative hydrostatic pressure gradient (e.g., suction through a straw).

The bulk movement of substances through the conducting or vascular tissues of plants is called translocation. Do you remember studying cross sections of roots, stems and leaves of higher plants and studying the vascular system? The higher plants have highly specialised vascular tissues – xylem and phloem. Xylem is associated with translocation of mainly water, mineral salts, some organic nitrogen and hormones, from roots to the aerial parts of the plants. The phloem translocates a variety of organic and inorganic solutes, mainly from the leaves to other parts of the plants.

1.3.1 How do Plants Absorb Water?

We know that the roots absorb most of the water that goes into plants; obviously that is why we apply water to the soil and not on the leaves. The responsibility of absorption of water and minerals is more specifically the function of the root hairs that are present in millions at the tips of the roots. Root hairs are thin-walled slender extensions of root epidermal cells that greatly increase the surface area for

absorption. Water is absorbed along with mineral solutes, by the root hairs, purely by diffusion. Once water is absorbed by the root hairs, it can move deeper into root layers by two distinct pathways:

• apoplast pathway

• symplast pathway

The apoplast is the system of adjacent cell walls that is continuous throughout the plant, except at the casparian strips of the endodermis in the roots (Figure 1.6). The apoplastic movement of water occurs exclusively through the intercellular spaces and the walls of the cells. Movement through the apoplast does not involve crossing the cell membrane. This movement is dependent on the gradient. The apoplast does not provide any barrier to water movement and water movement is through mass flow. As water evaporates into the intercellular spaces or the atmosphere, tension develop in the continuous stream of water in the apoplast, hence mass flow of water occurs due to the adhesive and cohesive properties of water. The symplastic system is the system of interconnected protoplasts. Neighbouring cells are connected through cytoplasmic strands that extend through plasmodesmata. During symplastic movement, the water travels through the cells – their cytoplasm; intercellular movement is through the plasmodesmata. Water has to enter the cells through the cell membrane, hence the movement is relatively slower. Movement is again down a potential gradient. Symplastic movement may be aided by cytoplasmic streaming. You may have observed cytoplasmic streaming in cells of the Hydrilla leaf; the movement of chloroplast due to streaming is easily visible. Most of the water flow in the roots occurs via the apoplast since the cortical cells are loosely packed, and hence offer no resistance to water movement. However, the inner boundary of the cortex, the endodermis, is impervious to water because of a band of suberised matrix called the casparian strip. Water molecules are unable to penetrate the layer, so they are directed to wall regions that are not suberised, into the cells proper through the membranes. The water then moves through the symplast and again crosses a membrane to reach the cells of the xylem. The movement of water through the root layers is ultimately symplastic in the endodermis. This is the only way water and other solutes can enter the vascular cylinder. Once inside the xylem, water is again free to move between cells as well as through them. In young roots, water enters directly into the xylem vessels and/or tracheids. These are non-living conduits and so are parts of the apoplast. The path of water and mineral ions into the root vascular system is summarised in Figure 1.7. Some plants have additional structures associated with them that help in water (and mineral) absorption. A mycorrhiza is a symbiotic association of a fungus with a root system. The fungal filaments form a network around the young root or they penetrate the root cells. The hyphae have a very large surface area that absorb mineral ions and water from the soil from a much larger volume of soil that perhaps a root cannot do. The

fungus provides minerals and water to the roots, in turn the roots provide sugars and N-containing compounds to the mycorrhizae. Some plants have an obligate association with the mycorrhizae. For example, Pinus seeds cannot germinate and establish without the presence of mycorrhizae.

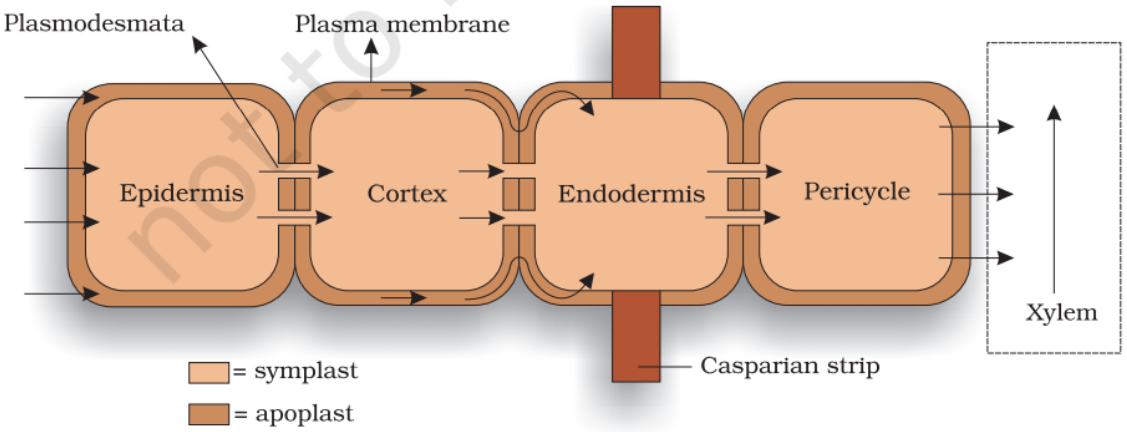

Figure 1.6 Pathway of water movement in the root

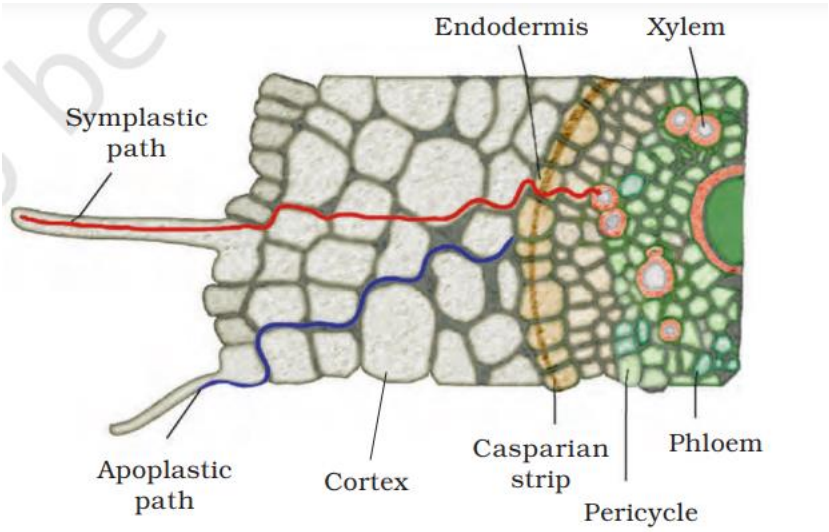

Figure 1.7 Symplastic and apoplastic pathways of water and ion absorption and movement in roots

1.3.2 Water Movement up a Plant

We looked at how plants absorb water from the soil, and move it into the vascular tissues. We now have to try and understand how this water is transported to various parts of the plant. Is the water movement active, or is it still passive? Since the water has to be moved up a stem against gravity, what provides the energy for this?

1.3.2.1 Root Pressure As various ions from the soil are actively transported into the vascular tissues of the roots, water follows (its potential gradient) and increases the pressure inside the xylem. This positive pressure is called root pressure, and can be responsible for pushing up water to small heights in the stem. How can we see that root pressure exists? Choose a small soft-stemmed plant and on a day, when there is plenty of atmospheric moisture, cut the stem horizontally near the base with a sharp blade, early in the morning. You will soon see drops of solution ooze out of the cut stem; this comes out due to the positive root pressure. If you fix a rubber tube to the cut stem as a sleeve you can actually collect and measure the rate of exudation, and also determine the composition of the exudates. Effects of root pressure is also observable at night and early morning when evaporation is low, and excess water collects in the form of droplets around special openings of veins near the tip of grass blades, and leaves of many herbaceous parts. Such water loss in its liquid phase is known as guttation. Root pressure can, at best, only provide a modest push in the overall process of water transport. They obviously do not play a major role in water movement up tall trees. The greatest contribution of root pressure may be to re-establish the continuous chains of water molecules in the xylem which often break under the enormous tensions created by transpiration. Root pressure does not account for the majority of water transport; most plants meet their need by transpiratory pull.

1.3.2.2 Transpiration pull Despite the absence of a heart or a circulatory system in plants, the upward flow of water through the xylem in plants can achieve fairly high rates, up to 15 metres per hour. How is this movement accomplished? A long standing question is, whether water is ‘pushed’ or ‘pulled’ through the plant. Most researchers agree that water is mainly ‘pulled’ through the plant, and that the driving force for this process is transpiration from the leaves. This is referred to as the cohesion-tension-transpiration pull model of water transport. But, what generates this transpirational pull? Water is transient in plants. Less than 1 per cent of the water reaching the leaves is used in photosynthesis and plant growth. Most of it is lost through the stomata in the leaves. This water loss is known as transpiration. You have studied transpiration in an earlier class by enclosing a healthy plant in polythene bag and observing the droplets of water formed inside the bag. You could also study water loss from a leaf using cobalt chloride paper, which turns colour on absorbing water.

1.4 TRANSPIRATION Transpiration is the evaporative loss of water by plants. It occurs mainly through stomata (sing. : stoma). Besides the loss of water vapour in transpiration, exchange of oxygen and carbon dioxide in the leaf also occurs through these stomata. Normally stomata are open in the day time and close during the night. The immediate cause of the opening or closing of stomata is a change in the turgidity of the guard cells. The inner wall of each guard cell, towards the pore or stomatal aperture, is thick and elastic. When turgidity increases within the two guard cells flanking each stomatal aperture or pore, the thin outer walls bulge out and force the inner walls into a crescent

shape. The opening of the stoma is also aided due to the orientation of the microfibrils in the cell walls of the guard cells. Cellulose microfibrils are oriented radially rather than longitudinally making it easier for the stoma to open. When the guard cells lose turgor, due to water loss (or water stress) the elastic inner walls regain their original shape, the guard cells become flaccid and the stoma closes. Usually the lower surface of a dorsiventral (often dicotyledonous) leaf has a greater number of stomata while in an isobilateral (often monocotyledonous) leaf they are about equal on both surfaces. Transpiration is affected by several external factors: temperature, light, humidity, wind speed. Plant factors that affect transpiration include number and distribution of stomata, per cent of open stomata, water status of the plant, canopy structure etc.

The transpiration driven ascent of xylem sap depends mainly on the following physical properties of water:

• Cohesion – mutual attraction between water molecules.

• Adhesion – attraction of water molecules to polar surfaces (such as the surface of tracheary elements).

• Surface Tension – water molecules are attracted to each other in the liquid phase more than to water in the gas phase.

These properties give water high tensile strength, i.e., an ability to resist a pulling force, and high capillarity, i.e., the ability to rise in thin tubes. In plants capillarity is aided by the small diameter of the tracheary elements – the tracheids and vessel elements. The process of photosynthesis requires water. The system of xylem vessels from the root to the leaf vein can supply the needed water. But what force does a plant use to move water molecules into the leaf parenchyma cells where they are needed? As water evaporates through the stomata, since the thin film of water over the cells is continuous, it results in pulling of water, molecule by molecule, into the leaf from the xylem. Also, because of lower concentration of water vapour in the atmosphere as compared to the substomatal cavity and intercellular spaces, water diffuses into the surrounding air. This creates a ‘pull’ (Figure 1.9). Measurements reveal that the forces generated by transpiration can create pressures sufficient to lift a xylem sized column of water over 130 metres high.

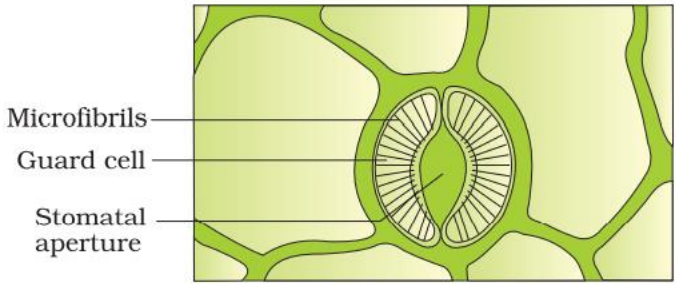

Figure. 1.8 A stomatal aperture with guard cells

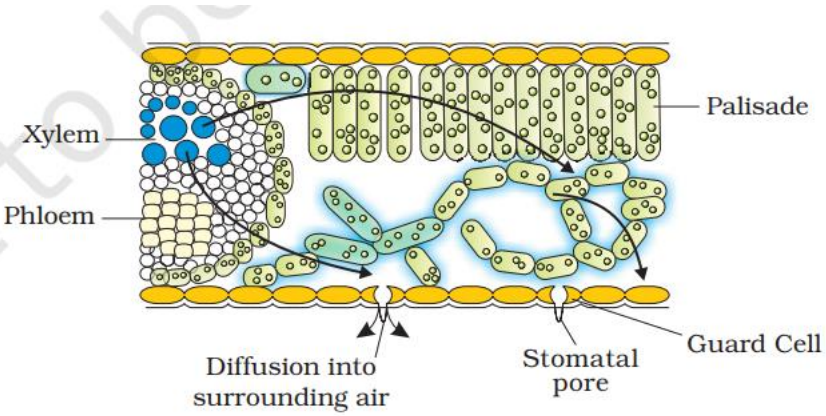

Figure. 1.9 Water movement in the leaf. Evaporation from the leaf sets up a pressure gradient between the outside air and the air spaces of the leaf. The gradient is transmitted into the photosynthetic cells and on the water-filled xylem in the leaf vein.

1.4.1 Transpiration and Photosynthesis – a Compromise Transpiration has more than one purpose; it

• creates transpiration pull for absorption and transport of plants

• supplies water for photosynthesis

• transports minerals from the soil to all parts of the plant

• cools leaf surfaces, sometimes 10 to 15 degrees, by evaporative cooling

• maintains the shape and structure of the plants by keeping cells turgid.

An actively photosynthesising plant has an insatiable need for water. Photosynthesis is limited by available water which can be swiftly depleted by transpiration. The humidity of rainforests is largely due to this vast cycling of water from root to leaf to atmosphere and back to the soil. The evolution of the C4 photosynthetic system is probably one of the strategies for maximising the availability of CO2 while minimising water loss. C4 plants are twice as efficient as C3 plants in terms of fixing carbon

dioxide (making sugar). However, a C4 plant loses only half as much water as a C3 plant for the same amount of CO2 fixed.

1.5 UPTAKE AND TRANSPORT OF MINERAL NUTRIENTS

Plants obtain their carbon and most of their oxygen from CO2 in the atmosphere. However, their remaining nutritional requirements are obtained from water and minerals in the soil.

1.5.1 Uptake of Mineral Ions Unlike water, all minerals cannot be passively absorbed by the roots. Two factors account for this: (i) minerals are present in the soil as charged particles (ions) which cannot move across cell membranes and (ii) the concentration of minerals in the soil is usually lower than the concentration of minerals in the root. Therefore, most minerals must enter the root by active absorption into the cytoplasm of epidermal cells. This needs energy in the form of ATP. The active uptake of ions is partly responsible for the water potential gradient in roots, and therefore for the uptake of water by osmosis. Some ions also move into the epidermal cells passively. Ions are absorbed from the soil by both passive and active transport. Specific proteins in the membranes of root hair cells actively pump ions from the soil into the cytoplasms of the epidermal cells. Like all cells, the endodermal cells have many transport proteins embedded in their plasma membrane; they let some solutes cross the membrane, but not others. Transport proteins of endodermal cells are control points, where a plant adjusts the quantity and types of solutes that reach the xylem. Note that the root endodermis because of the layer of suberin has the ability to actively transport ions in one direction only.

1.5.2 Translocation of Mineral Ions After the ions have reached xylem through active or passive uptake, or a combination of the two, their further transport up the stem to all parts of the plant is through the transpiration stream. The chief sinks for the mineral elements are the growing regions of the plant, such as the apical and lateral meristems, young leaves, developing flowers, fruits and seeds, and the storage organs. Unloading of mineral ions occurs at the fine vein endings through diffusion and active uptake by these cells. Mineral ions are frequently remobilised, particularly from older, senescing parts. Older dying leaves export much of their mineral content to younger leaves. Similarly, before leaf fall in decidous plants, minerals are removed to other parts. Elements most readily mobilised are phosphorus, sulphur, nitrogen and potassium. Some elements that are structural components like calcium are not remobilised. An analysis of the xylem exudates shows that though some of the nitrogen travels as inorganic ions, much of it is carried in the organic form as amino acids and related compounds. Similarly, small amounts of P and S are carried as organic compounds. In addition, small amount of exchange of materials does take place between xylem and phloem. Hence,

it is not that we can clearly make a distinction and say categorically that xylem transports only inorganic nutrients while phloem transports only organic materials, as was traditionally believed.

1.6 PHLOEM TRANSPORT: FLOW FROM SOURCE TO SINK

Food, primarily sucrose, is transported by the vascular tissue phloem from a source to a sink. Usually the source is understood to be that part of the plant which synthesises the food, i.e., the leaf, and sink, the part that needs or stores the food. But, the source and sink may be reversed depending on the season, or the plant's needs. Sugar stored in roots may be mobilised to become a source of food in the early spring when the buds of trees, act as sink; they need energy for growth and development of the photosynthetic apparatus. Since the source-sink relationship is variable, the direction of movement in the phloem can be upwards or downwards, i.e., bi-directional. This contrasts with that of the xylem where the movement is always unidirectional, i.e., upwards. Hence, unlike one-way flow of water in transpiration, food in phloem sap can be transported in any required direction so long as there is a source of sugar and a sink able to use, store or remove the sugar. Phloem sap is mainly water and sucrose, but other sugars, hormones and amino acids are also transported or translocated through phloem.

1.6.1 The Pressure Flow or Mass Flow Hypothesis

The accepted mechanism used for the translocation of sugars from source to sink is called the pressure flow hypothesis. (see Figure 1.10). As glucose is prepared at the source (by photosynthesis) it is converted to sucrose (a dissacharide). The sugar is then moved in the form of sucrose into the companion cells and then into the living phloem sieve tube cells by active transport. This process of loading at the source produces a hypertonic condition in the phloem. Water in the adjacent xylem moves into the phloem by osmosis. As osmotic pressure builds up the phloem sap will move to areas of lower pressure. At the sink osmotic pressure must be reduced. Again active transport is necessary to move the sucrose out of the phloem sap and into the cells which will use the sugar – converting it into energy, starch, or cellulose. As sugars are removed, the osmotic pressure decreases and water moves out of the phloem. To summarise, the movement of sugars in the phloem begins at the source, where sugars are loaded (actively transported) into a sieve tube. Loading of the phloem sets up a water potential gradient that facilitates the mass movement in the phloem. Phloem tissue is composed of sieve tube cells, which form long columns with holes in their end walls called sieve plates. Cytoplasmic strands pass through the holes in the sieve plates, so forming continuous filaments. As hydrostatic pressure in the sieve tube of phloem increases, pressure flow begins, and the sap moves through the phloem. Meanwhile, at the sink, incoming sugars are actively transported out of the phloem and removed as complex carbohydrates. The loss of solute produces a high water potential in

the phloem, and water passes out, returning eventually to xylem. A simple experiment, called girdling, was used to identify the tissues through which food is transported. On the trunk of a tree a ring of bark up to a depth of the phloem layer, can be carefully removed. In the absence of downward movement of food the portion of the bark above the ring on the stem becomes swollen after a few weeks. This simple experiment shows that phloem is the tissue responsible for translocation of food; and that transport takes place in one direction, i.e., towards the roots. This experiment can be performed by you easily.

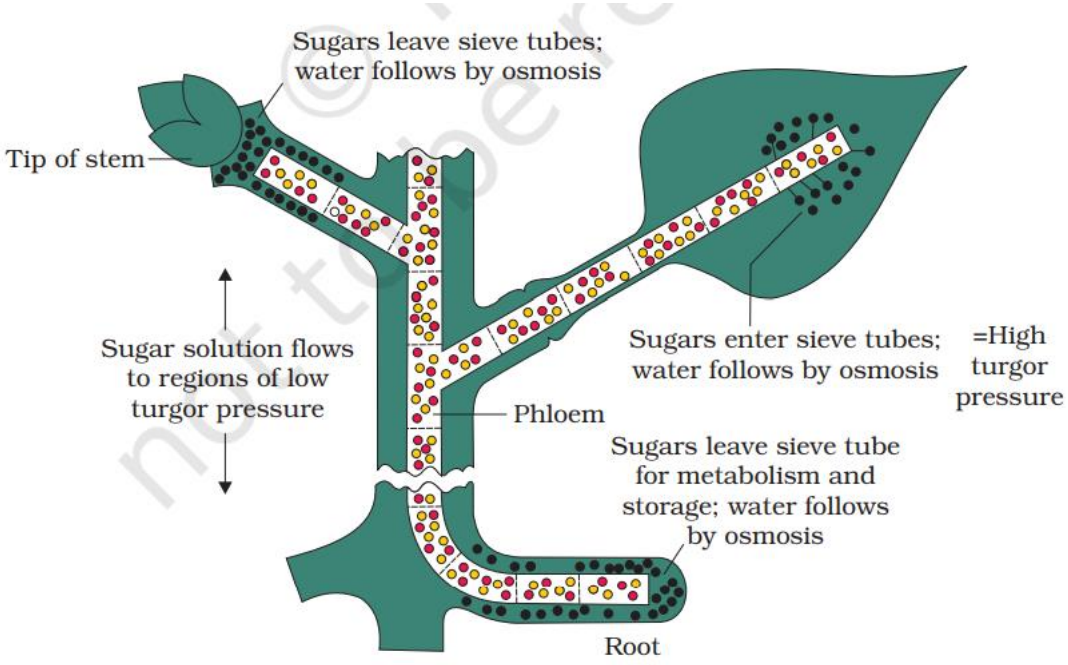

Figure 1.10 Diagrammatic presentation of mechanism of translocation

Summary

Plants obtain a variety of inorganic elements (ions) and salts from their surroundings especially from water and soil. The movement of these nutrients from environment into the plant as well as from one plant cell to another plant cell essentially involves movement across a cell membrane. Transport across cell membrane can be through diffusion, facilitated transport or active transport. Water and minerals absorbed by roots are transported by xylem and the organic material synthesised in the leaves is transported to other parts of plant through phloem. Passive transport (diffusion, osmosis) and active transport are the two modes of nutrient transport across cell membranes in living organisms. In passive transport, nutrients move across the membrane by diffusion, without any use of energy as it is always down the concentration gradient and hence entropy driven. This diffusion of substances depends on their size, solubility in water or organic solvents. Osmosis is the special type of diffusion of water across a selectively permeable membrane which depends on pressure gradient

and concentration gradient. In active transport, energy in the form of ATP is utilised to pump molecules against a concentration gradient across membranes. Water potential is the potential energy of water molecules which helps in the movement of water. It is determined by solute potential and pressure potential. The osmotic behaviour of cells depends on the surrounding solution. If the surrounding solution of the cell is hypertonic, it gets plasmolysed. The absorption of water by seeds and drywood takes place by a special type of diffusion called imbibition. In higher plants, there is a vascular system comprising of xylem and phloem, responsible for translocation. Water minerals and food cannot be moved within the body of a plant by diffusion alone. They are therefore, transported by a mass flow system – movement of substance in bulk from one point to another as a result of pressure differences between the two points. Water absorbed by root hairs moves into the root tissue by two distinct pathways, i.e., apoplast and symplast. Various ions, and water from soil can be transported upto a small height in stems by root pressure. Transpiration pull model is the most acceptable to explain the transport of water. Transpiration is the loss of water in the form of vapours from the plant parts through stomata. Temperature, light, humidity, wind speed and number of stomata affect the rate of transpiration. Excess water is also removed through tips of leaves of plants by guttation. Phloem is responsible for transport of food (primarily) sucrose from the source to the sink. The translocation in phloem is bi-directional; the source-sink relationship is variable. The translocation in phloem is explained by the pressure flow hypothesis.

Note: this notes are taken from NCERT

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DEPARTMENT OF BIOTECHNOLOGY

UNIT – II– PLANT BIOCHEMISTRY– SBC3201

CHLOROPLAST AND ITS ROLE IN PHOTOSYNTHESIS

Plants form the basis of all life on earth and are known as producers. Plant cells contain structures known as plastids which are absent in animal cells. These plastids are double-membraned cell organelles which play a primary role in the manufacturing and storing of food. There are three types of plastids –

- Chromoplasts- They are the colour plastids, found in all flowers, fruits and are mainly responsible for their distinctive colours.
- Chloroplasts- They are green coloured plastids, which comprises green-coloured pigments within the plant cell and are called as the chlorophyll.
- Leucoplasts- They are colourless plastids and are mainly used for the storage of starch, lipids, and proteins within the plant cell.

Chloroplasts- Definition, Structure, Functions and Diagram

- The word *chloroplast is* derived from the Greek words *chloros*, which means green, and *plastes*, which means "the one who forms".
- Chloroplasts are a type of membrane-bound plastids that contain a network of membranes embedded into a liquid matrix and harbor the photosynthetic pigment called chlorophyll.
- It is this pigment that imparts a green color to plant parts and serves to capture light energy.
- Chloroplasts can be found in the cells of the mesophyll in plant leaves.
- There are usually 30-40 per mesophyll cells.

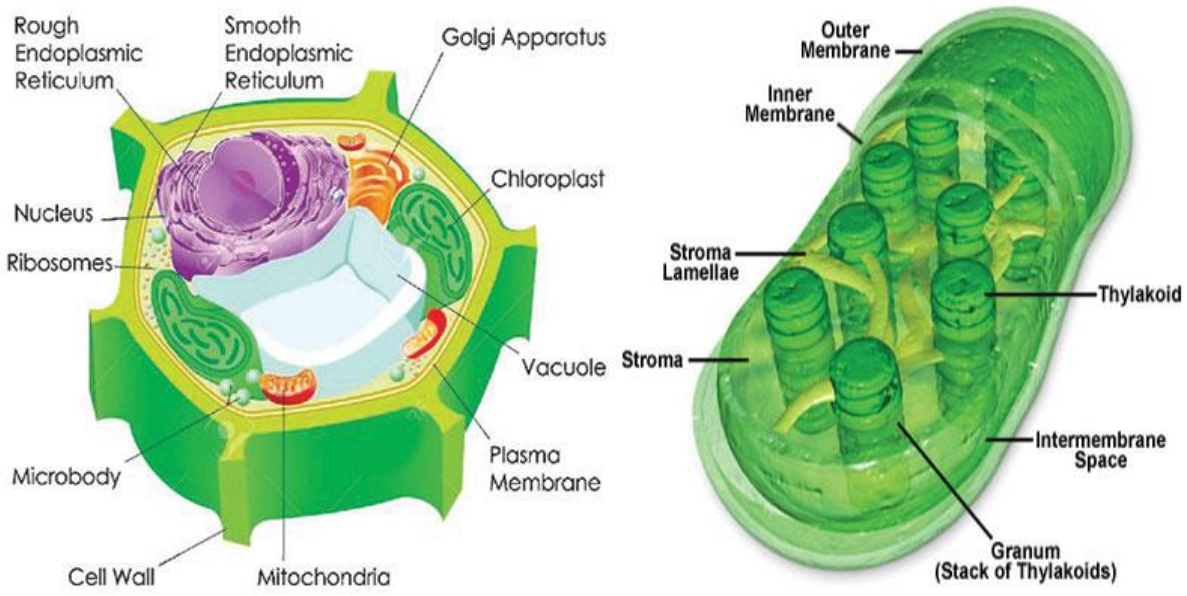


Figure: Diagram of Chloroplasts

Structure of Chloroplasts

- Chloroplasts found in higher plants are generally biconvex or planoconvex shaped.
- In different plants, however, chloroplasts may have different shapes, varying from spheroid, filamentous saucer-shaped, discoid or ovoid-shaped.
- They can be found in the cells of the mesophyll in plant leaves. They are vesicular and have a colorless center.
- The average size of the chloroplast is 4-6 Âµ in diameter and 1-3 Âµ in thickness.

The chloroplast has an inner and outer membrane with an empty intermediate space in between. Inside the chloroplast are stacks of thylakoids, called grana, as well as stroma, the dense fluid inside of the chloroplast. These thylakoids contain the chlorophyll that is necessary for the plant to go through photosynthesis. The space the chlorophyll fills is called the thylakoid space.

A chloroplast thus has the following parts:

1. **Envelope (Outer membrane**)

It is a semi-porous membrane and is permeable to small molecules and ions, which diffuses easily. The outer membrane is not permeable to larger proteins.

2. **Intermembrane Space**

It is usually a thin inter-membrane space about 10-20 nanometers and it is present between the outer and the inner membrane of the chloroplast.

3. **Inner membrane**

The inner membrane of the chloroplast forms a border to the stroma. It regulates the passage of materials in and out of the chloroplast. In addition to regulation activity, fatty acids, lipids, and carotenoids are synthesized in the inner chloroplast membrane.

4. **Stroma**

Stroma is an alkaline, aqueous fluid that is protein-rich and is present within the inner membrane of the chloroplast. The space outside the thylakoid space is called the stroma. The chloroplast DNA chloroplast ribosomes and the thylakoid system, starch granules and many proteins are found floating around the stroma.

5. **Thylakoid System**

The thylakoid system is suspended in the stroma. The thylakoid system is a collection of membranous sacs called thylakoids. The chlorophyll is found in the thylakoids and is the sight for the process of light reactions of photosynthesis to happen. The thylakoids are arranged in stacks known as grana. Each granum contains around 10-20 thylakoids.

Peripheral Reticulum

The chloroplasts of certain plants contain an additional set of membranous tubules called peripheral reticulum that originates from the inner membrane of the envelope. Tiny vesicles bud off from the inner membrane of the chloroplast and assemble to form the tubules of the peripheral reticulum.

Functions of Chloroplasts

- Chloroplasts are the sites for photosynthesis, which comprises a set of light-dependent and light-independent reactions to harness solar energy and convert it into chemical energy.

- The components of chloroplast participate in several regulatory functions of the cell as well as in photorespiration.
- Chloroplasts also provide diverse metabolic activities for plant cells, including the synthesis of fatty acids, membrane lipids, isoprenoids, tetrapyrroles, starch, and hormones.
- Plants lack specialized immune cells—all plant cells participate in the plant response.
- The chloroplasts with the **nucleus** and cell membrane and **ER** are the key organelles of pathogen defense.
- Chloroplasts can serve as cellular sensors.

Functions of Chloroplast

Following are the important chloroplast function:

- The most important function of the chloroplast is to synthesize food by the process of photosynthesis.
- Absorbs light energy and converts it into chemical energy.
- Chloroplast has a structure called chlorophyll which functions by trapping the solar energy and used for the synthesis of food in all green plants.
- Produces NADPH and molecular oxygen (O_2) by photolysis of water.
- Produces ATP – Adenosine triphosphate by the process of photosynthesis.
- The carbon dioxide (CO2) obtained from the air is used to generate carbon and sugar during the Calvin Cycle or dark reaction of photosynthesis

Photosynthesis Notes

The following points describe the significance and relationship of photosynthesis to life on earth.

1. Photosynthesis begins the carbon cycle by fixing CO_2 (carbon dioxide in the atmosphere)

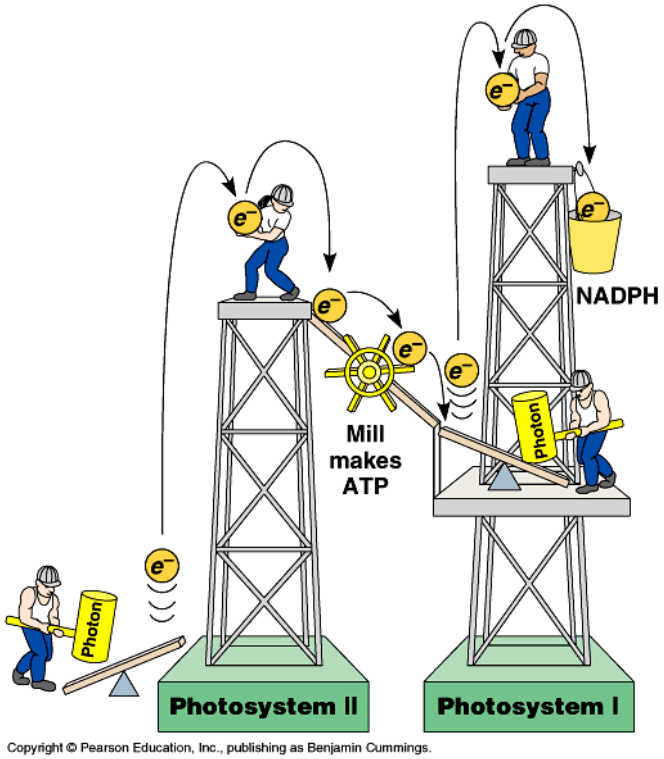
2. It has 2 major metabolic components: the light reactions and the "dark" reactions

3. The overall equation for photosynthesis is:

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

4. This equation leaves out many important details such as:

a. where does the O_2 gas come from? (water)
b. how is light energy converted into chemical bond energy?



5. The oxygen released as a by-product has a major impact on the bioshpere. Today's atmosphere would not have 21% oxygen if not for photosynthesis.

6. Three kingdoms have photosynthetic autotrophs (producers)

a. monera - cyanobacteria
b. protista - algae

c. plants

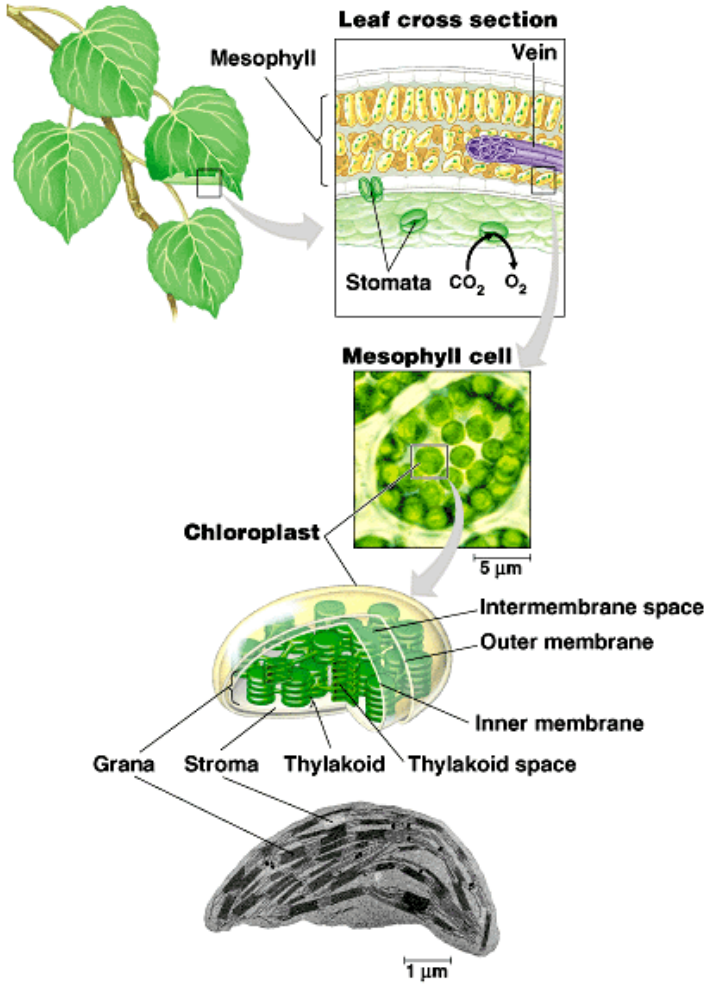
7. Photosynthesis is endergonic △G = +686 kcal/mole

Photosynthesis - The Light Reactions

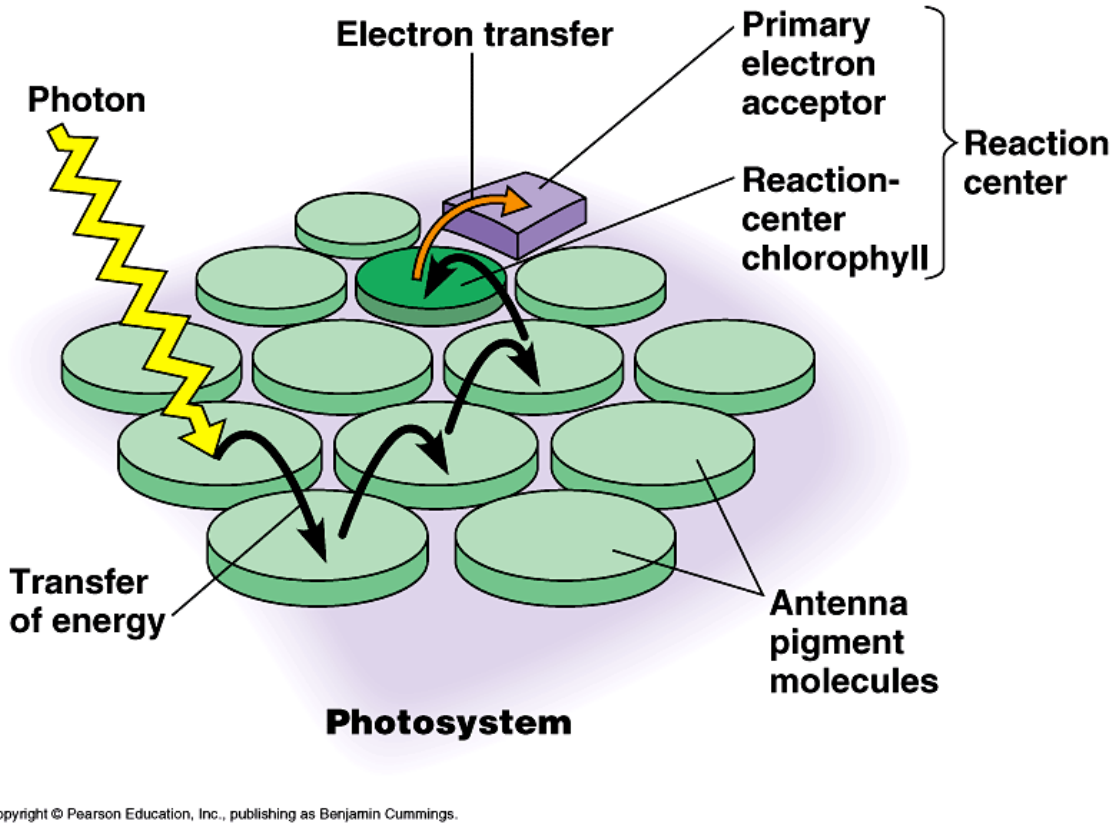
The light reactions of photosynthesis convert radiant (sunlight) energy into the potential chemical energy found between the carbon, hydrogen, and oxygen bonds in sugar (glucose).

1. Photosynthesis uses most of the energy in sunlight except green wavelengths (color that's reflected)

2. The light reactions occur on thylakoid membranes in plants (similar membranes in protista or cyanobacteria)

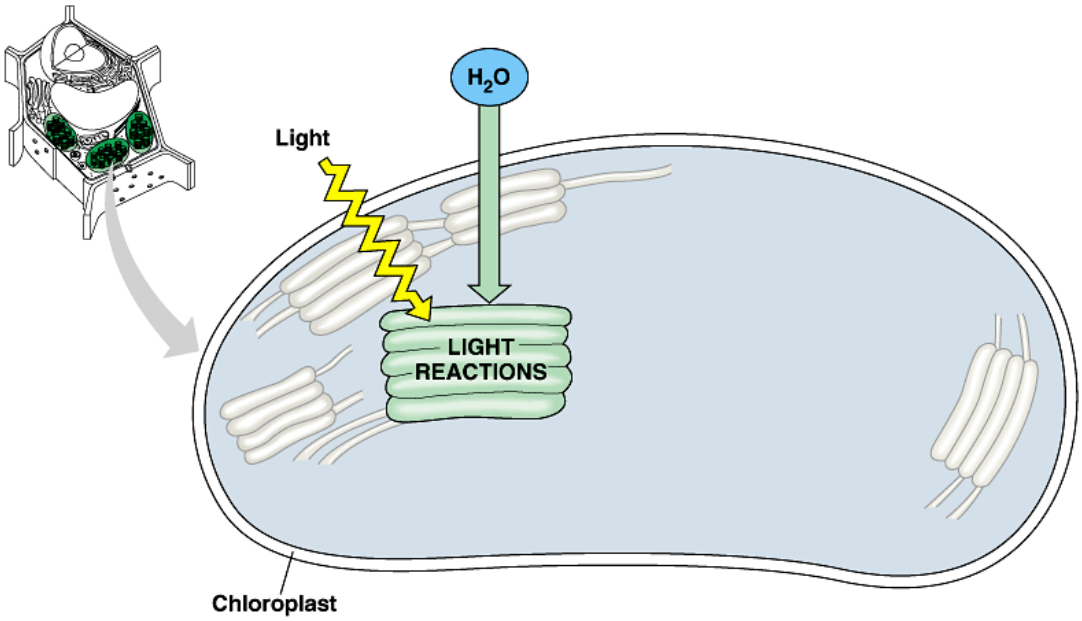


3. Chlorophyll molecules contain an atom of Mg (magnesium metal) which loses electrons and becomes oxidized by light. The electrons are accepted by an adjacent electron transport chain. 



4. There are 2 photosystems: photosystem I (cyclic) and photosystem II (noncyclic)

5. Photosystem II (P680 - noncyclic)



- After chlorophyll has lost electrons, enzyme X splits water (photolysis) releasing electrons to reduce chlorophyll.
- Two H^+ ions made available by photolysis are pumped into the thylakoid lumen by PQ a mobile carrier in the electron transport chain embedded in the thylakoid membrane.
- Electrons do not stop until they pass through photosystem I and finally reduce $NADP^+$ to $NADPH_2$.

6. Photosystem I (P700 - cyclic)

Photosystem I can act on its own, sending electrons to FD (ferrodoxin) and back to P700 to pump H^+ and make ATP.

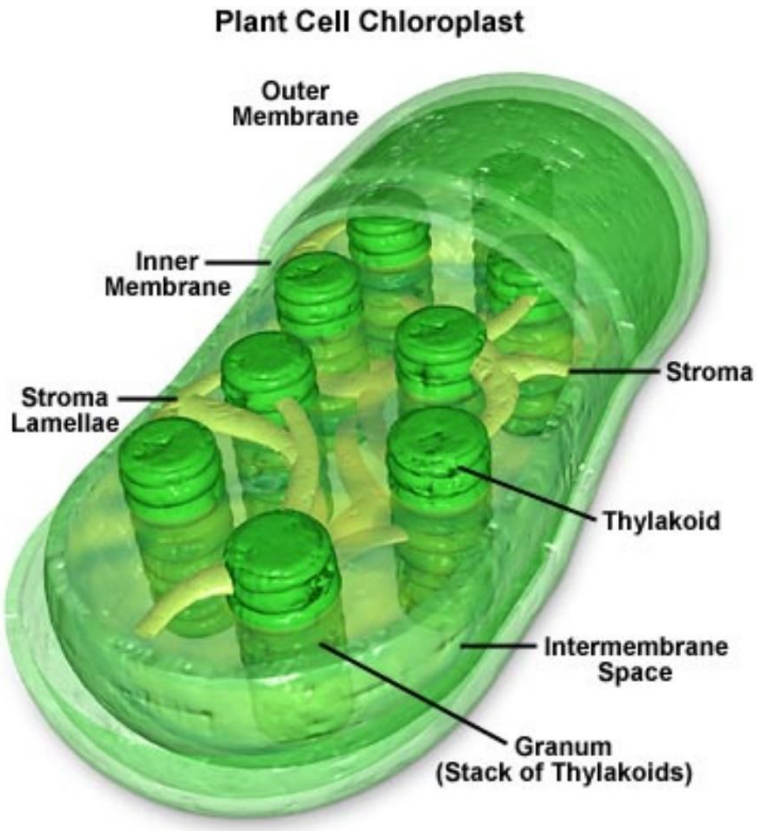
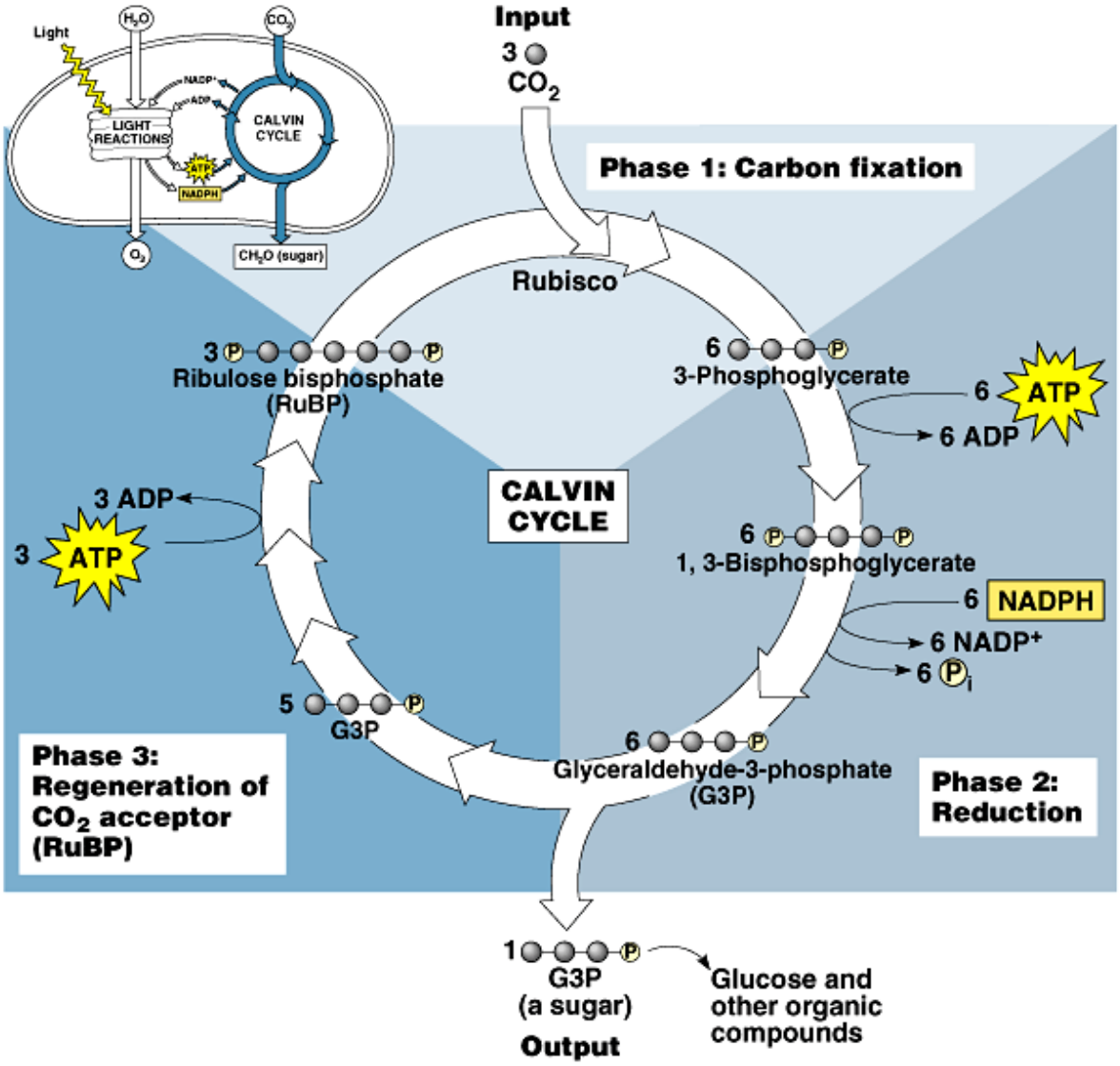


Figure 1

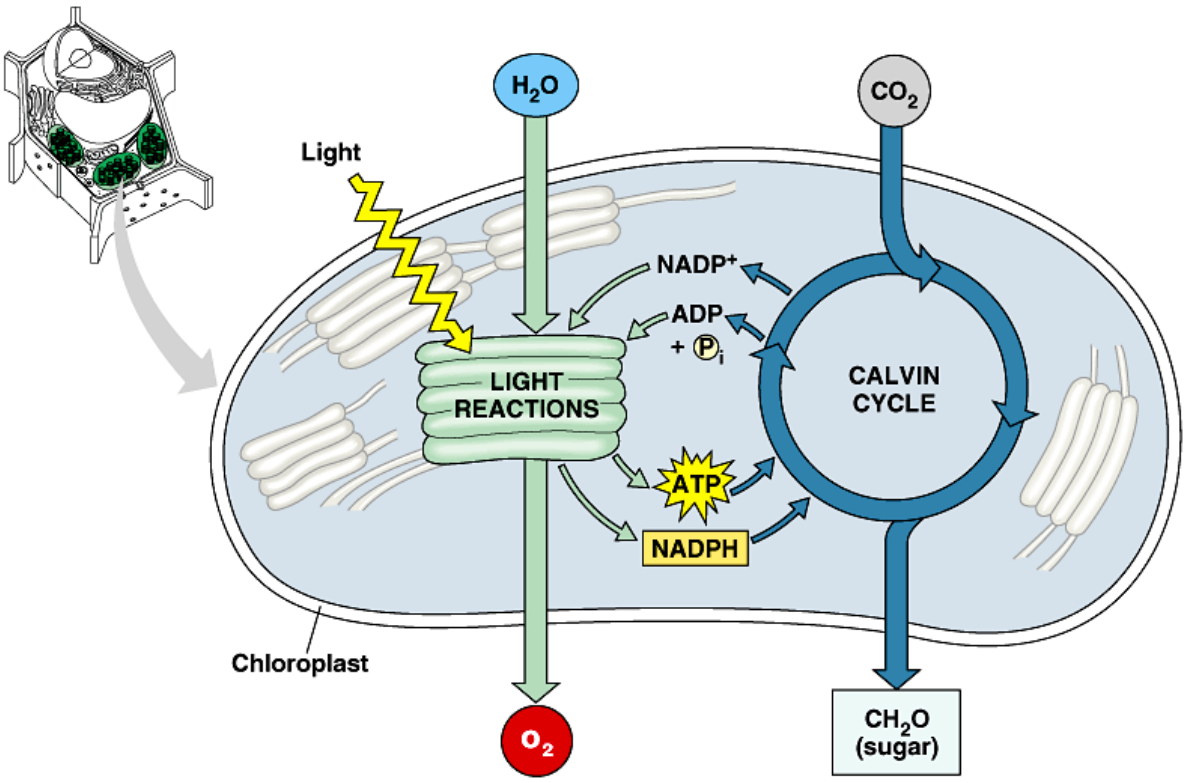
The Dark Reactions (Light Independent)

What you need to know about the dark reactions.

1. They can occur with or without light.
2. They occur in the stroma of chloroplasts.
3. The dark reaction requires ATP and NADPH -- products of the light reaction.
4. In addition the dark reaction requires CO_2, RuBP (a 5-carbon sugar called ribulose bisphosphate), and rubisco (the most plentiful enzyme on earth.
5. One glucose molecule is formed only after 6 turns of the Calvin cycle.



6. The 6-carbon molecule first formed by rubisco is unstable and is quickly converted to 2 molecules of PGAL (glyceraldehyde-3-phosphate also known as G3P). PGAL can be returned to the Calvin cycle as RuBP or used in glycolysis and several other anabolic processes to make amino acids or lipids.



The light-dependent reactions

Introduction

Plants and other photosynthetic organisms are experts at collecting solar energy, thanks to the light-absorbing pigment molecules in their leaves. But what happens to the light energy that is absorbed? We don’t see plant leaves glowing like light bulbs, but we also know that energy can't just disappear (thanks to the First Law of Thermodynamics).

As it turns out, some of the light energy absorbed by pigments in leaves is converted to a different form: chemical energy. Light energy is converted to chemical energy during the first stage of photosynthesis, which involves a series of chemical reactions known as the light-dependent reactions.

In this article, we'll explore the light-dependent reactions as they take place during photosynthesis in plants. We'll trace how light energy is absorbed by pigment molecules, how reaction center pigments pass excited electrons to an electron transport chain, and how the energetically "downhill" flow of electrons leads to synthesis of ATP and NADPH. These molecules store energy for use in the next stage of photosynthesis: the Calvin cycle.

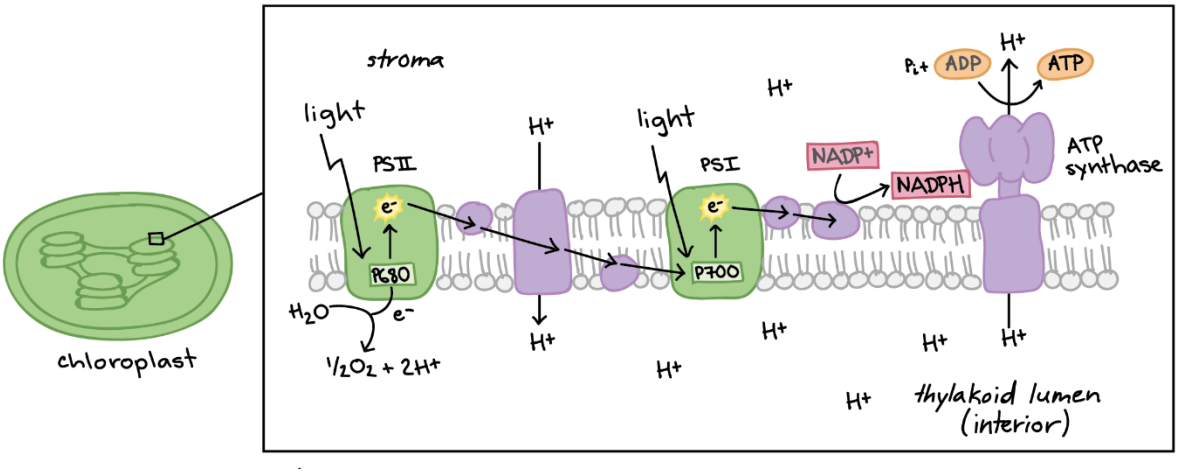
Overview of the light-dependent reactions

Before we get into the details of the light-dependent reactions, let's step back and get an overview of this remarkable energy-transforming process.

The **light-dependent reactions** use light energy to make two molecules needed for the next stage of photosynthesis: the energy storage molecule ATP and the reduced electron carrier NADPH. In plants, the light reactions take place in the thylakoid membranes of organelles called chloroplasts.

Photosystems, large complexes of proteins and pigments (light-absorbing molecules) that are optimized to harvest light, play a key role in the light reactions. There are two types of photosystems: photosystem I (PSI) and photosystem II (PSII).

Both photosystems contain many pigments that help collect light energy, as well as a special pair of chlorophyll molecules found at the core (reaction center) of the photosystem. The special pair of **photosystem I** is called **P700**, while the special pair of **photosystem II** is called **P680**.



In a process called **non-cyclic photophosphorylation** (the "standard" form of the light-dependent reactions), electrons are removed from water and passed through PSII and PSI before ending up in NADPH. This process requires light to be absorbed twice, once in each photosystem, and it makes ATP . In fact, it's called photophosphorylation because it involves using light energy (*photo*) to make ATP from ADP (*phosphorylation*). Here are the basic steps:

- **Light absorption in PSII.** When light is absorbed by one of the many pigments in photosystem II, energy is passed inward from pigment to pigment until it reaches the reaction center. There, energy is transferred to P680, boosting an electron to a high energy level. The high-energy electron is passed to an acceptor molecule and replaced with an electron from water. This splitting of water releases O2 we breathe.
- **ATP synthesis.** The high-energy electron travels down an electron transport chain, losing energy as it goes. Some of the released energy drives pumping of H+ ions from the stroma into the thylakoid interior, building a gradient. As H+ ions flow down their

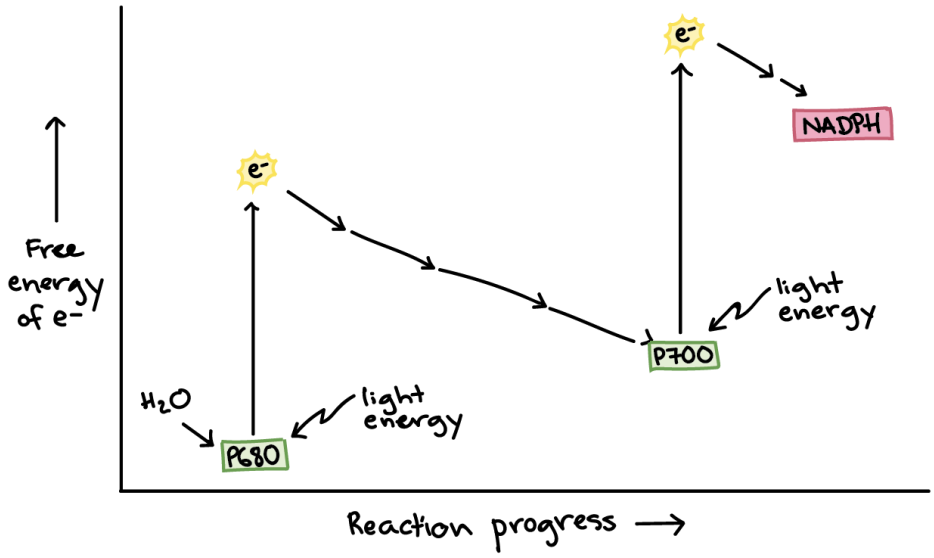
gradient and into the stroma, they pass through ATP synthase, driving ATP production in a process known as **chemiosmosis**.

- **ATP synthesis.** The high-energy electron travels down an electron transport chain, losing energy as it goes. Some of the released energy drives pumping of H+ from the stroma into the thylakoid interior, building a gradient. (H+ ions from the splitting of water also add to the gradient.) As H+ ions flow down their gradient and into the stroma, they pass through ATP synthase, driving ATP production in a process known as **chemiosmosis**.
- **Light absorption in PSI.** The electron arrives at photosystem I and joins the P700 special pair of chlorophylls in the reaction center. When light energy is absorbed by pigments and passed inward to the reaction center, the electron in P700 is boosted to a very high energy level and transferred to an acceptor molecule. The special pair's missing electron is replaced by a new electron from PSII (arriving via the electron transport chain).
- **NADPH formation.** The high-energy electron travels down a short second leg of the electron transport chain. At the end of the chain, the electron is passed to NADP+ (along with a second electron from the same pathway) to make NADPH.

The net effect of these steps is to convert light energy into chemical energy in the form of ATP and NADPH. The ATP and NADPH from the light-dependent reactions are used to make sugars in the next stage of photosynthesis, the Calvin cycle. In another form of the light reactions, called **cyclic photophosphorylation**, electrons follow a different, circular path and only ATP (no NADPH) is produced.

[More on cyclic photophosphorylation]

It's important to realize that the electron transfers of the light-dependent reactions are driven by, and indeed made possible by, the absorption of energy from light. In other words, the transfers of electrons from PSII to PSI, and from PSI to NADPH, are only energetically "downhill" (energy-releasing, and thus spontaneous) because electrons in P680 and P700 are boosted to very high energy levels by absorption of energy from light.

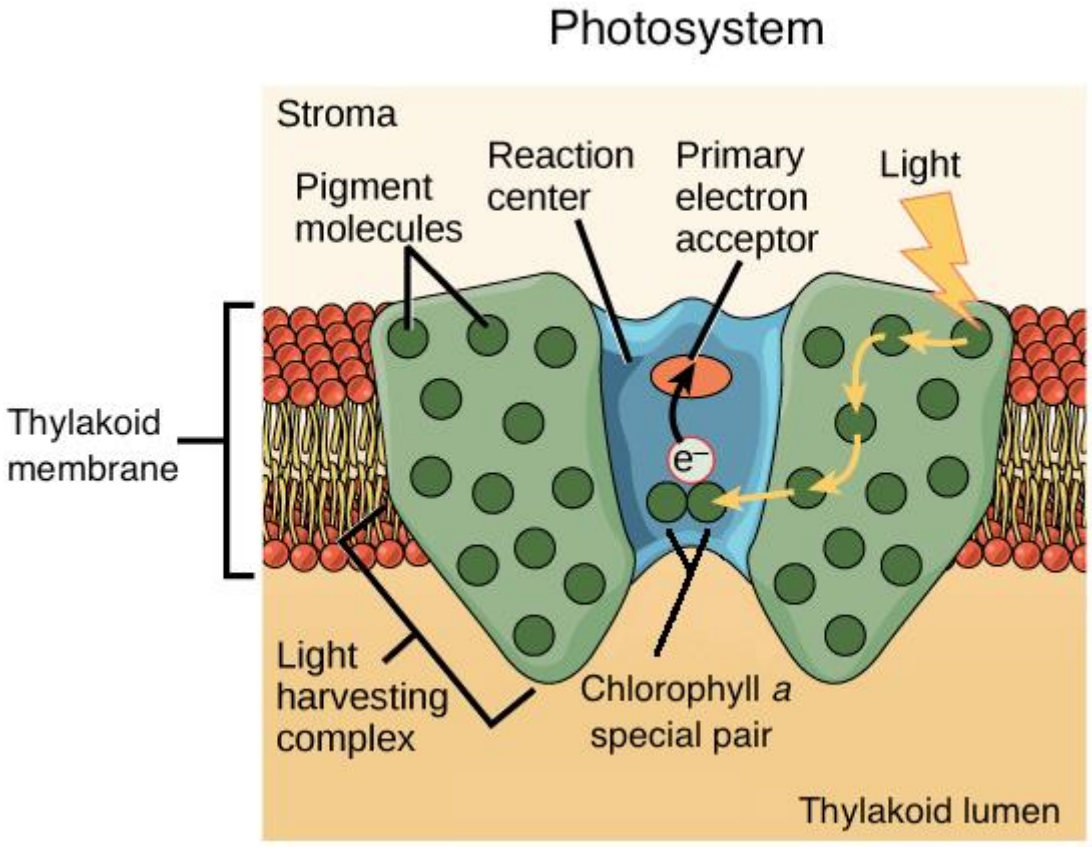


What is a photosystem?

Photosynthetic pigments, such as chlorophyll *a*, chlorophyll *b*, and carotenoids, are light-harvesting molecules found in the thylakoid membranes of chloroplasts. As mentioned above, pigments are organized along with proteins into complexes called **photosystems**. Each photosystem has **light-harvesting complexes** that contain proteins, 300300300-400400400 chlorophylls, and other pigments. When a pigment absorbs a photon, it is raised to an excited state, meaning that one of its electrons is boosted to a higher-energy orbital.

Most of the pigments in a photosystem act as an energy funnel, passing energy inward to a main reaction center. When one of these pigments is excited by light, it transfers energy to a neighboring pigment through direct electromagnetic interactions in a process called **resonance energy transfer**. The neighbor pigment, in turn, can transfer energy to one of its own neighbors, with the process repeating multiple times. In these transfers, the receiving molecule cannot require more energy for excitation than the donor, but may require less energy (i.e., may absorb light of a longer wavelength)^66start superscript, 6, end superscript.

Collectively, the pigment molecules collect energy and transfer it towards a central part of the photosystem called the **reaction center**.



he reaction center of a photosystem contains a unique pair of chlorophyll *a* molecules, often called **special pair** (actual scientific name—that's how special it is!). Once energy reaches the special pair, it will no longer be passed on to other pigments through resonance energy transfer. Instead, the special pair can actually lose an electron when excited, passing it to another molecule in the complex called the **primary electron acceptor**. With this transfer, the electron will begin its journey through an electron transport chain.

Photosystem I vs. photosystem II

There are two types of photosystems in the light-dependent reactions, **photosystem II (PSII)** and **photosystem I (PSI)**. PSII comes first in the path of electron flow, but it is named as second because it was discovered after PSI. (Thank you, historical order of discovery, for yet another confusing name!)

Here are some of the key differences between the photosystems:

- **Special pairs.** The chlorophyll *a* special pairs of the two photosystems absorb different wavelengths of light. The PSII special pair absorbs best at 680 nm, while the PSI special absorbs best at 700 nm. Because of this, the special pairs are called **P680** and **P700**, respectively.
- **Primary acceptor**. The special pair of each photosystem passes electrons to a different primary acceptor. The primary electron acceptor of PSII is pheophytin, an organic molecule that resembles chlorophyll, while the primary electron acceptor of PSI is a chlorophyll called A0.
- **Source of electrons**. Once an electron is lost, each photosystem is replenished by electrons from a different source. The PSII reaction center gets electrons from water, while the PSI reaction center is replenished by electrons that flow down an electron transport chain from PSII.

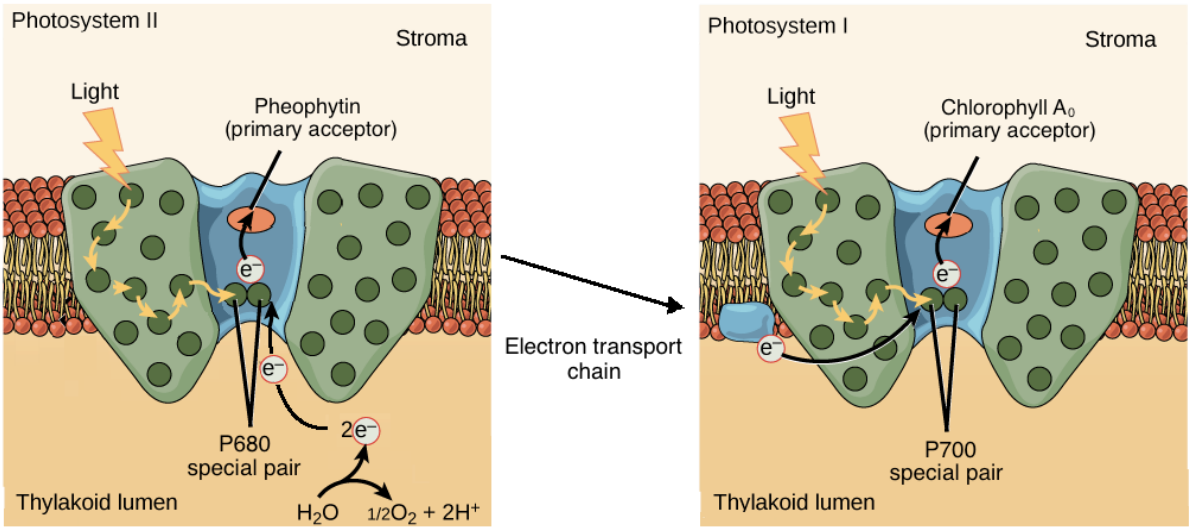


Image modified from "The Light-Dependent Reactions of Photosynthesis: Figure 7," by OpenStax College, Biology (CC BY 4.0.

During the light-dependent reactions, an electron that's excited in PSII is passed down an electron transport chain to PSI (losing energy along the way). In PSI, the electron is excited again and passed down the second leg of the electron transport chain to a final electron acceptor. Let’s trace the path of electrons in more detail, starting when they're excited by light energy in PSII.

Photosystem II

When the P680 special pair of photosystem II absorbs energy, it enters an excited (high-energy) state. Excited P680 is a good electron donor and can transfer its excited electron to the primary electron acceptor, pheophytin. The electron will be passed on through the first leg of the photosynthetic **electron transport chain** in a series of redox, or electron transfer, reactions.

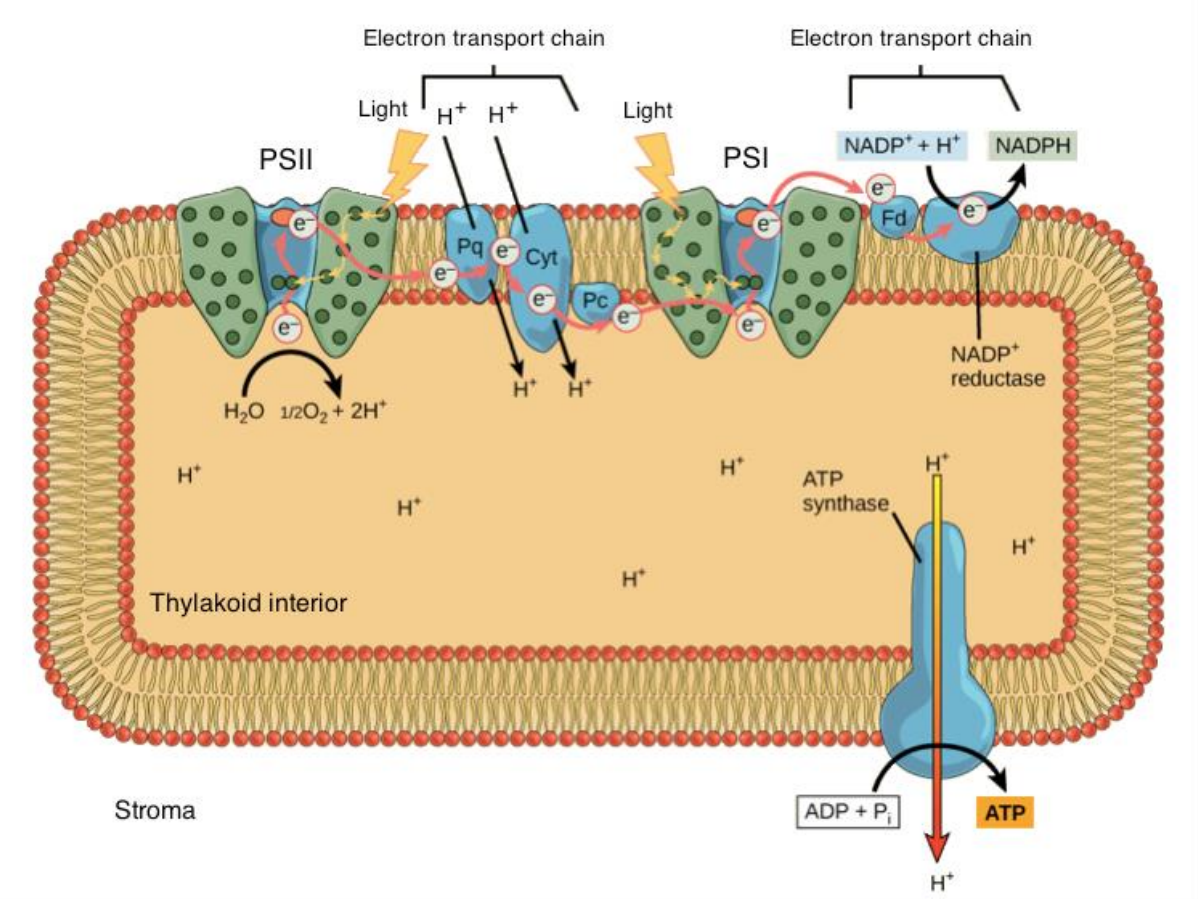
After the special pair gives up its electron, it has a positive charge and needs a new electron. This electron is provided through the splitting of water molecules, a process carried out by a portion of PSII called the manganese center 9. The positively charged P680 can pull electrons off of water (which doesn't give them up easily) because it's extremely "electron-hungry."

When the manganese center splits water molecules, it binds two at once, extracting four electrons, releasing four H+ ions, and producing a molecule of O2. About 101010 percent of the oxygen is used by mitochondria in the leaf to support oxidative phosphorylation. The remainder escapes to the atmosphere where it is used by aerobic organisms (such as us!) to support respiration.

Electron transport chains and photosystem I

When an electron leaves PSII, it is transferred first to a small organic molecule (plastoquinone, Pq), then to a cytochrome complex (Cyt), and finally to a copper-containing protein called plastocyanin (Pc). As the electron moves through this electron transport chain, it goes from a higher to a lower energy level, releasing energy. Some of the energy is used to pump protons (H+) from the stroma (outside of the thylakoid) into the thylakoid interior.

This transfer of H+, along with the release of H+ from the splitting of water, forms a proton gradient that will be used to make ATP (as we'll see shortly).



The light-dependent reactions involve two photosytems (II and I) and an electron transport chain that are all embedded in the thylakoid membrane. Light that is harvested from PSII causes an excited electron of the chlorophyll *a* special pair to be passed down an electron transport chain (Pq, Cyt, and Pc) to PSI. The electron lost from the chlorophyll *a* special pair is replenished by splitting water.

The passing of the electron in the first part of the electron transport chain causes protons to be pumped from the stroma to the thylakoid lumen. A concentration gradient formed (with a higher concentration of protons in the thylakoid lumen than in the stroma). Protons diffuse out of the thylakoid lumen through the enzyme, ATP synthase, producing ATP in the process.

Once the electron reaches PSI, it joins its chlorophyll *a* special pair and re-excited by the absorption of light. It proceeds down a second part of the electron transport chain (Fd and NADP+ reductase) and reduces NADP+ to form NADPH. The electron lost from the chlorophyll *a* special pair is replenished by electrons flowing from PSII.

Image modified from "The Light-Dependent Reactions of Photosynthesis: Figure 8," by OpenStax College, Biology (CC BY 4.0.

Once an electron has gone down the first leg of the electron transport chain, it arrives at PSI, where it joins the chlorophyll *a* special pair called P700. Because electrons have lost energy prior to their arrival at PSI, they must be re-energized through absorption of another photon.

Excited P700 is a very good electron donor, and it sends its electron down a short electron transport chain. In this series of reactions, the electron is first passed to a protein called ferredoxin (Fd), then transferred to an enzyme called **NADP+ reductase**. NADP+ reductase transfers electrons to the electron carrier NADP+ to make NADPH. NADPH will travel to the Calvin cycle, where its electrons are used to build sugars from carbon dioxide.

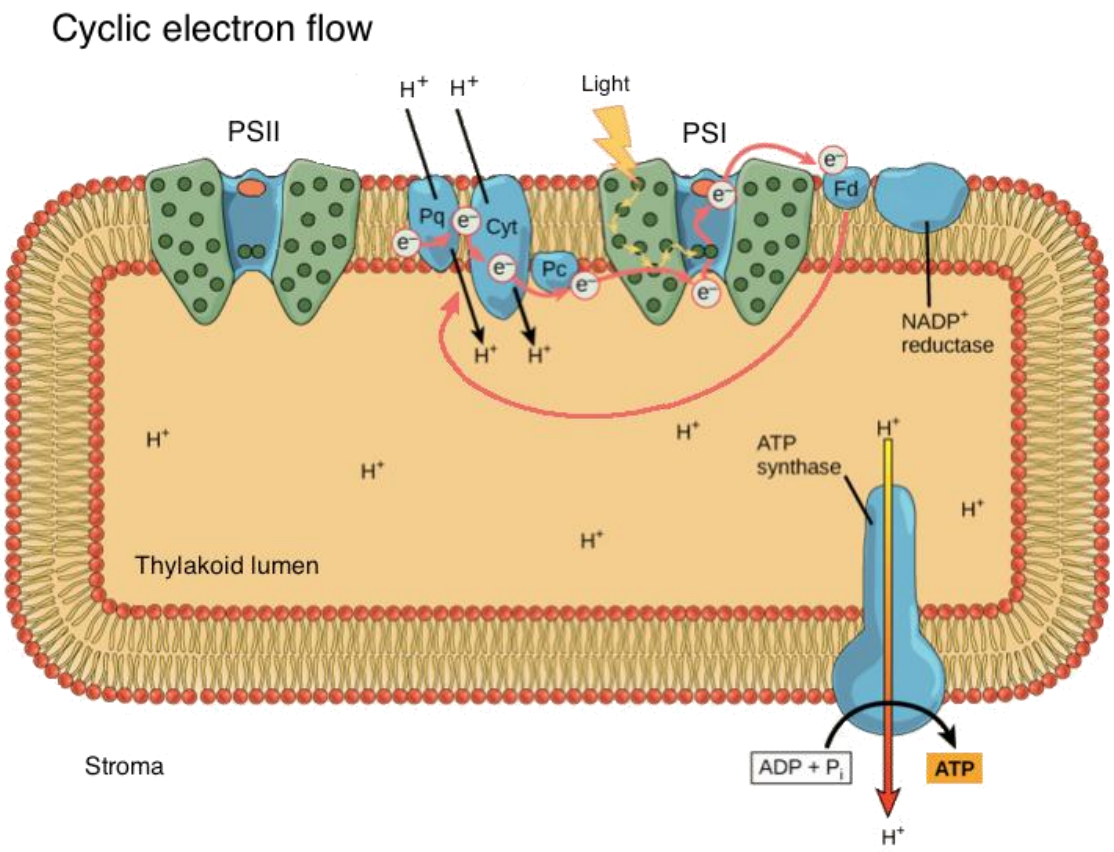
The other ingredient needed by the Calvin cycle is ATP, and this too is provided by the light reactions. As we saw above, H+ ions build inside the thylakoid interior and make a concentration gradient. Protons "want" to diffuse back down the gradient and into the stroma, and their only route of passage is through the enzyme **ATP synthase**. ATP synthase harnesses the flow of protons to make ATP from ADP and phosphate (P*i*). This process of making ATP using energy stored in a chemical gradient is called **chemiosmosis**.

Some electrons flow cyclically

The pathway above is sometimes called **linear photophosphorylation**. That's because electrons travel in a line from water through PSII and PSI to NADPH. (*Photophosphorylation* = light-driven synthesis of ATP.)

In some cases, electrons break this pattern and instead loop back to the first part of the electron transport chain, repeatedly cycling through PSI instead of ending up in NADPH. This is called **cyclic photophosphorylation**.

After leaving PSI, cyclically flowing electrons travel back to the cytochrome complex (Cyt) or plastoquinone (Pq) in the first leg of the electron transport chain {10,11}. The electrons then flow down the chain to PSI as usual, driving proton pumping and the production of ATP. The cyclic pathway does not make NADPH, since electrons are routed away from NADP+ reductase.



In cyclic electron flow, electrons are repeatedly cycled though PSI. After an electron in PSI is excited and passed to ferredoxin, it is passed back to the cytochrome complex in the first part of the electron transport chain. Cyclically flowing electrons result in the production of ATP (because protons are pumped into the thylakoid lumen), but do not result in the production of NADPH (because electrons are not passed to NADP+ reductase).

Image modified from "The Light-Dependent Reactions of Photosynthesis: Figure 8," by OpenStax College, Biology (CC BY 4.0.

Why does the cyclic pathway exist? At least in some cases, chloroplasts seem to switch from linear to cyclic electron flow when the ratio of NADPH to NADP+ is too high (when too little NADP+ is available to accept electrons). In addition, cyclic electron flow may be common in photosynthetic cell types with especially high ATP needs (such as the sugar-synthesizing bundle-sheath cells of plants that carry out C4. Finally, cyclic electron flow may play a photoprotective role, preventing excess light from damaging photosystem proteins and promoting repair of light-induced damage.

Calvin cycle

Introduction

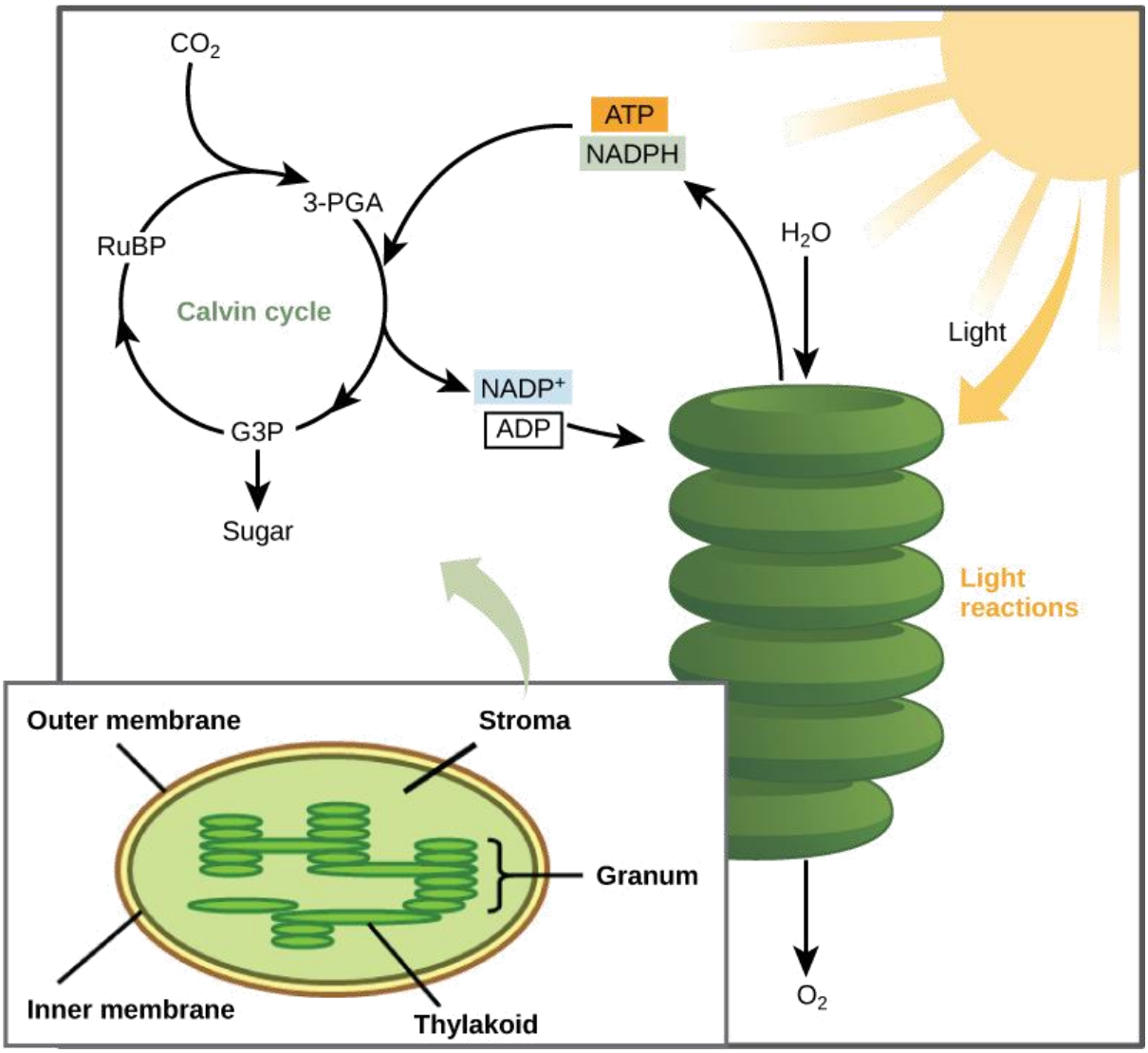
You, like all organisms on Earth, are a carbon-based life form. In other words, the complex molecules of your amazing body are built on carbon backbones. You might already know that you're carbon-based, but have you ever wondered where all of that carbon comes from?

As it turns out, the atoms of carbon in your body were once part of carbon dioxide (CO_2) molecules in the air. Carbon atoms end up in you, and in other life forms, thanks to the second stage of photosynthesis, known as the **Calvin cycle** (or the **light-independent reactions**).

Overview of the Calvin cycle

In plants, carbon dioxide (CO_2) enters the interior of a leaf via pores called stomata and diffuses into the stroma of the chloroplast—the site of the **Calvin cycle** reactions, where sugar is synthesized. These reactions are also called the **light-independent** reactions because they are not directly driven by light.

In the Calvin cycle, carbon atoms from CO_2 are **fixed** (incorporated into organic molecules) and used to build three-carbon sugars. This process is fueled by, and dependent on, ATP and NADPH from the light reactions. Unlike the light reactions, which take place in the thylakoid membrane, the reactions of the Calvin cycle take place in the stroma (the inner space of chloroplasts).



This illustration shows that ATP and NADPH produced in the light reactions are used in the Calvin cycle to make sugar.

Image credit: "The Calvin cycle: Figure 1," by OpenStax College, Concepts of Biology CC BY 4.0

Reactions of the Calvin cycle

The Calvin cycle reactions can be divided into three main stages: carbon fixation, reduction, and regeneration of the starting molecule.

Here is a general diagram of the cycle:

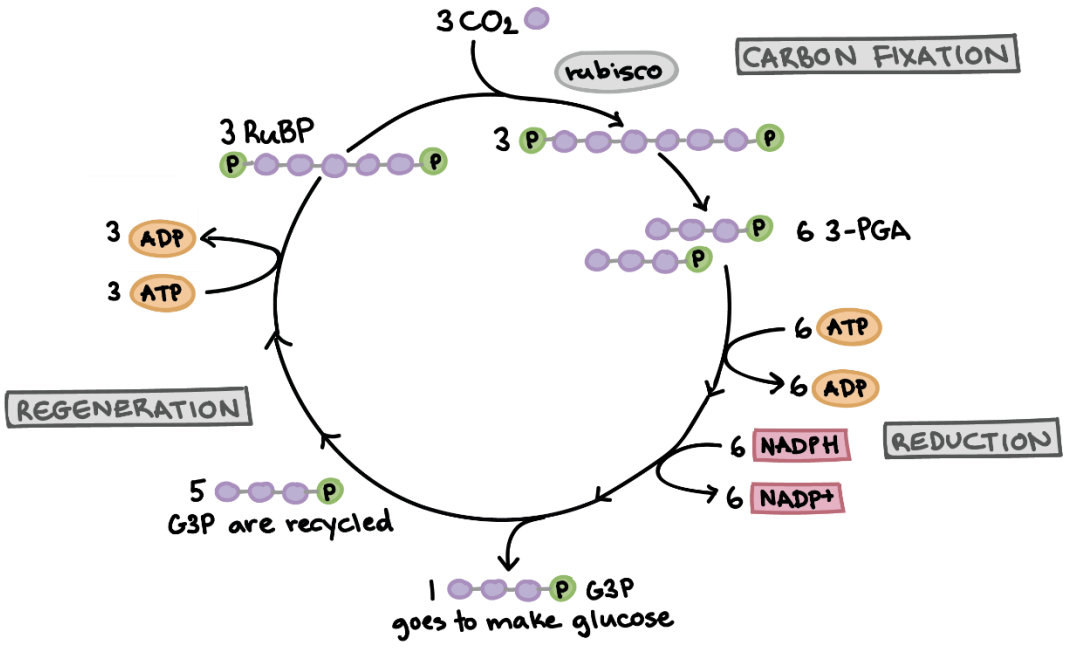


Diagram of the Calvin cycle, illustrating how the fixation of three carbon dioxide molecules allows one net G3P molecule to be produced (that is, allows one G3P molecule to leave the cycle).

CO2, molecules combine with three molecules of the five-carbon acceptor molecule (RuBP), yielding three molecules of an unstable six-carbon compound that splits to form six molecules of a three-carbon compound (3-PGA). This reaction is catalyzed by the enzyme rubisco.

In the second stage, six ATP and six NADPH are used to convert the six 3-PGA molecules into six molecules of a three-carbon sugar (G3P). This reaction is considered a reduction because NADPH must donate its electrons to a three-carbon intermediate to make G3P.

3. **Regeneration.** One G3P molecule leaves the cycle and will go towards making glucose, while five G3Ps must be recycled to regenerate the RuBP acceptor. Regeneration involves a complex series of reactions and requires ATP.

[See a diagram that shows the molecular structures]

1. **Carbon fixation.** A CO2 molecule combines with a five-carbon acceptor molecule, ribulose-1,5-bisphosphate (**RuBP**). This step makes a six-carbon compound that splits into two molecules of a three-carbon compound, 3-phosphoglyceric acid (3-PGA). This reaction is catalyzed by the enzyme RuBP carboxylase/oxygenase, or **rubisco**. [Details of this step]

2. **Reduction.** In the second stage, ATP and NADPH are used to convert the 3-PGA molecules into molecules of a three-carbon sugar, glyceraldehyde-3-phosphate (**G3P**). This stage gets its name because NADPH donates electrons to, or **reduces**, a three-carbon intermediate to make G3P.
   [Details of this step]

3. **Regeneration.** Some G3P molecules go to make glucose, while others must be recycled to regenerate the RuBP acceptor. Regeneration requires ATP and involves a complex network of reactions, which my college bio professor liked to call the "carbohydrate scramble."

In order for one G3P to exit the cycle (and go towards glucose synthesis),
three CO_2 molecules must enter the cycle, providing three new atoms of fixed carbon. When three CO_2 molecules enter the cycle, six G3P molecules are made. One exits the cycle and is used to make glucose, while the other five must be recycled to regenerate three molecules of the RuBP acceptor.

Summary of Calvin cycle reactants and products

Three turns of the Calvin cycle are needed to make one G3P molecule that can exit the cycle and go towards making glucose. Let's summarize the quantities of key molecules that enter and exit the Calvin cycle as one net G3P is made. In three turns of the Calvin cycle:

- **Carbon.** 3 CO_2 combine with 3 RuBP acceptors, making 6 molecules of glyceraldehyde-3-phosphate (G3P).
  - 1 G3P molecule exits the cycle and goes towards making glucose.
  - 5 G3P molecules are recycled, regenerating 3 RuBP acceptor molecules.
- **ATP.** 9 ATP are converted to 9 ADP (6 during the fixation step, 3 during the regeneration step).
- **NADPH**. 6 NADPH are converted to 6 $NADP^+$ (during the reduction step).

A G3P molecule contains three fixed carbon atoms, so it takes two G3Ps to build a six-carbon glucose molecule. It would take six turns of the cycle, or 6 CO_2 , 18 ATP, and 12 NADPH, to produce one molecule of glucose.

C3, C4, and CAM plants

Key points:

- **Photorespiration** is a wasteful pathway that occurs when the Calvin cycle enzyme rubisco acts on oxygen rather than carbon dioxide.
- The majority of plants are C3 **plants**, which have no special features to combat photorespiration.
- C4 **plants** minimize photorespiration by separating initial CO2fixation and the Calvin cycle in space, performing these steps in different cell types.
- **Crassulacean acid metabolism** (**CAM**) plants minimize photorespiration and save water by separating these steps in time, between night and day.

Introduction

High crop yields are pretty important—for keeping people fed, and also for keeping economies running. If you heard there was a single factor that reduced the yield of wheat by 20% and the yield of soybeans by 36% in the United States, for instance, you might be curious to know what it was

As it turns out, the factor behind those (real-life) numbers is photorespiration. This wasteful metabolic pathway begins when rubisco, the carbon-fixing enzyme of the Calvin cycle, grabs O2 rather than CO2. It uses up fixed carbon, wastes energy, and tends to happens when plants close their stomata (leaf pores) to reduce water loss. High temperatures make it even worse.

Some plants, unlike wheat and soybean, can escape the worst effects of photorespiration. The C4 and CAM pathways are two adaptations—beneficial features arising by natural selection—that allow certain species to minimize photorespiration. These pathways work by ensuring that Rubisco always encounters high concentrations of CO2, making it unlikely to bind to O2.

In the rest of this article, we'll take a closer look at the C4 and CAM pathways and see how they reduce photorespiration.

C3 plants

A "normal" plant—one that doesn't have photosynthetic adaptations to reduce photorespiration—is called a C3 plant. The first step of the Calvin cycle is the fixation of carbon dioxide by rubisco, and plants that use only this "standard" mechanism of carbon fixation are called C3 plants, for the three-carbon compound (3-PGA) the reaction produces. About 85% of the plant species on the planet are C3 plants, including rice, wheat, soybeans and all trees.

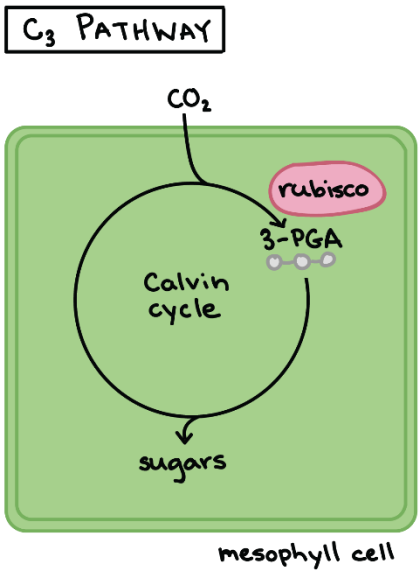


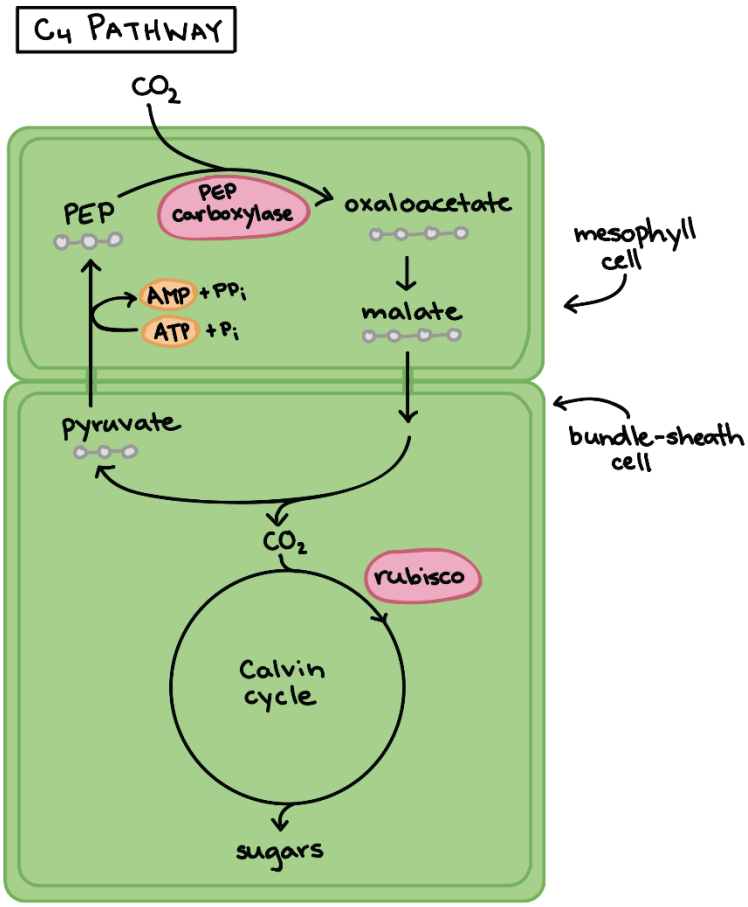
Image of the C3 pathway. Carbon dioxide enters a mesophyll cell and is fixed immediately by rubisco, leading to the formation of 3-PGA molecules, which contain three carbons.

C4 plants

In C4 plants, the light-dependent reactions and the Calvin cycle are physically separated, with the light-dependent reactions occurring in the mesophyll cells (spongy tissue in the middle of the leaf) and the Calvin cycle occurring in special cells around the leaf veins. These cells are called **bundle-sheath** cells.

To see how this division helps, let's look at an example of C4 photosynthesis in action. First, atmospheric CO2is fixed in the mesophyll cells to form a simple, 4-carbon organic acid (oxaloacetate). This step is carried out by a non-rubisco enzyme, PEP carboxylase, that has no tendency to bind O2. Oxaloacetate is then converted to a similar molecule, malate, that can be transported in to the bundle-sheath cells. Inside the bundle sheath, malate breaks

down, releasing a molecule of CO2. The CO2 is then fixed by rubisco and made into sugars via the Calvin cycle, exactly as in C3 photosynthesis.



In the C4 pathway, initial carbon fixation takes place in mesophyll cells and the Calvin cycle takes place in bundle-sheath cells. PEP carboxylase attaches an incoming carbon dioxide molecul to the three-carbon molecule PEP, producing oxaloacetate (a four-carbon molecule). The oxaloacetate is converted to malate, which travels out of the mesophyll cell and into a neighboring bundle-sheath. Inside the bundle sheath cell, malate is broken down to release CO2 which then enters the Calvin cycle. Pyruvate is also produced in this step and moves back into the mesophyll cell, where it is converted into PEP (a reaction that converts ATP and Pi into AMP and PPi).

This process isn't without its energetic price: ATP must be expended to return the three-carbon “ferry” molecule from the bundle sheath cell and get it ready to pick up another molecule of atmospheric CO2. However, because the mesophyll cells constantly pump CO2 into neighboring bundle-sheath cells in the form of malate, there’s always a high

concentration of CO_2 relative to O_2 right around rubisco. This strategy minimizes photorespiration.

The C4 pathway is used in about 3% of all vascular plants; some examples are crabgrass, sugarcane and corn. C4 plants are common in habitats that are hot, but are less abundant in areas that are cooler. In hot conditions, the benefits of reduced photorespiration likely exceed the ATP cost of moving CO_2 from the mesophyll cell to the bundle-sheath cell.

CAM plants

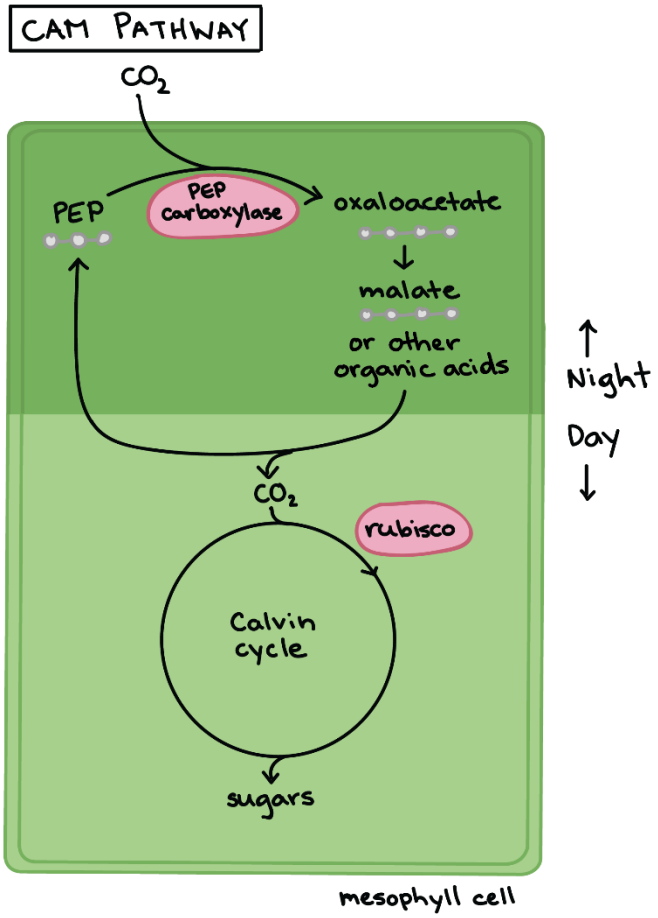
Some plants that are adapted to dry environments, such as cacti and pineapples, use the **crassulacean acid metabolism** (**CAM**) pathway to minimize photorespiration. This name comes from the family of plants, the Crassulaceae, in which scientists first discovered the pathway.

Image of a succulent.

Image credit: "Crassulaceae," by Guyon Morée (CC BY 2.0).

Instead of separating the light-dependent reactions and the use of CO2 in the Calvin cycle in space, CAM plants separate these processes in time. At night, CAM plants open their stomata, allowing CO2 to diffuse into the leaves. This CO2 is fixed into oxaloacetate by PEP carboxylase (the same step used by C4 plants), then converted to malate or another type of organic acid.

The organic acid is stored inside vacuoles until the next day. In the daylight, the CAM plants do not open their stomata, but they can still photosynthesize. That's because the organic acids are transported out of the vacuole and broken down to release CO2 which enters the Calvin cycle. This controlled release maintains a high concentration of CO2 around rubisco.



CAM plants temporally separate carbon fixation and the Calvin cycle. Carbon dioxide diffuses into leaves during the night (when stomata are open) and is fixed into oxaloacetate by PEP carboxylase, which attaches the carbon dioxide to the three-carbon molecule PEP. The oxaloacetate is converted to another organic acid, such as malate. The organic acid is stored

until the next day and is then broken down, releasing carbon dioxide that can be fixed by rubisco and enter the Calvin cycle to make sugars.

The CAM pathway requires ATP at multiple steps (not shown above), so like C4 photosynthesis, it is not an energetic "freebie."However, plant species that use CAM photosynthesis not only avoid photorespiration, but are also very water-efficient. Their stomata only open at night, when humidity tends to be higher and temperatures are cooler, both factors that reduce water loss from leaves. CAM plants are typically dominant in very hot, dry areas, like deserts.

Comparisons of C3, C4 and CAM plants

C3 , C4 and CAM plants all use the Calvin cycle to make sugars from CO2. These pathways for fixing CO2 have different advantages and disadvantages and make plants suited for different habitats. The C3 mechanism works well in cool environments, while C4 and CAM plants are adapted to hot, dry areas.

Both the C4 and CAM pathways have evolved independently over two dozen times, which suggests they may give plant species in hot climates a significant evolutionary advantage

Type	Separation of initial CO_2 fixation and Calvin cycle	Stomata open	Best adapted to
C_3	No separation	Day	Cool, wet environments
C_4	Between mesophyll and bundle-sheath cells (in space)	Day	Hot, sunny environments
CAM	Between night and day (in time)	Night	Very hot, dry environments

Photorespiration in C3 and C4 plants

As we all know, photosynthesis is a biochemical process of producing carbohydrates using light energy. The whole process is carried in two phases.

1. Photochemical phase – In the photochemical phase, ATP and NADPH are produced
2. Biosynthetic phase – In this phase, the final product glucose is formed.

Based on, how plants proceed in the biosynthetic phase, plants are further classified as C_3 and C_4 plants. Another factor which differentiates a C_4 plant from C_3 is photorespiration.

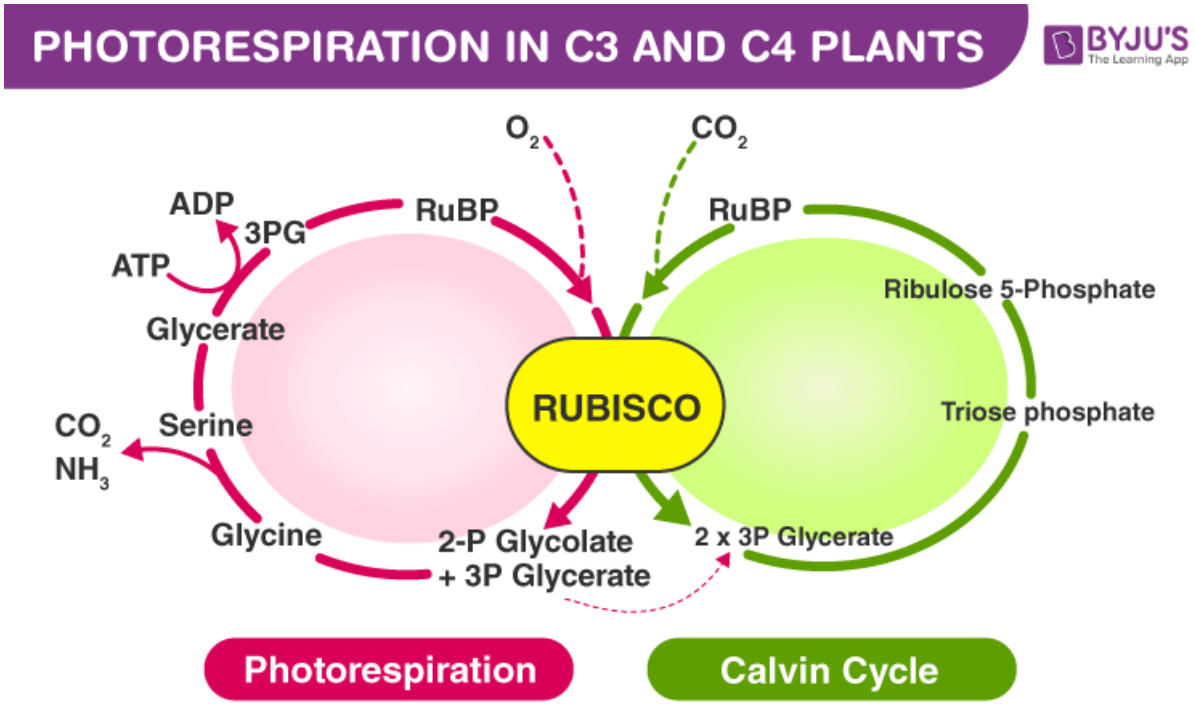
Photorespiration

Photorespiration is a respiratory process in many higher plants. This is also known as the oxidative photosynthetic, or C_2 photosynthesis or carbon cycle.

For a better understanding of photorespiration, let's recall the Calvin cycle, the first step of the biosynthetic phase in C_3 plants- Plants which use only the Calvin cycle for fixing the carbon dioxide.

The reaction in which carbon dioxide and water combine to give carbohydrates is termed as carbon fixation. Calvin cycle is the first step of carbon fixation where CO_2 combines with Ribulose-1 and 5-bisphosphate (RuBP) to form two molecules of 3 carbon acid called 3-phosphoglycericacid (PGA). The reaction is catalyzed by the most abundant enzyme in the world called RuBisCO (RuBP carboxylase-oxygenase). RuBisCO is the enzyme that has an affinity for both CO_2 and O_2 but has more affinity for CO_2. Hence, the binding of CO_2 and O_2 are competitive in nature and the concentration of the molecules in the atmosphere determines the winner. This is the basis for photorespiration in plants.

Sometimes in C_3 plants, RuBisCO binds to oxygen molecules and the reaction deviates from the regular metabolic pathway. The combination of RuBP and oxygen molecules leads to the formation of one molecule of phosphoglycerate and phosphoglycolate. This pathway is called photorespiration. During photorespiration, no sugar or ATP molecules are synthesized, but just CO_2 is released at the expense of ATP and the whole process is futile.



However, C_4 plants do not undergo photorespiration due to their special mechanism to increase the CO_2 level for enzyme binding. During the Hatch and Slack Pathway, the C_4 acid, oxaloacetic acid (OAA) breaks down to release CO_2. This ensures the high concentration of intercellular CO_2. Thus, in C_4 plants, RuBisCO is more active as a carboxylase enzyme rather than as oxygenase. This is why C_4 plants have better productivity.

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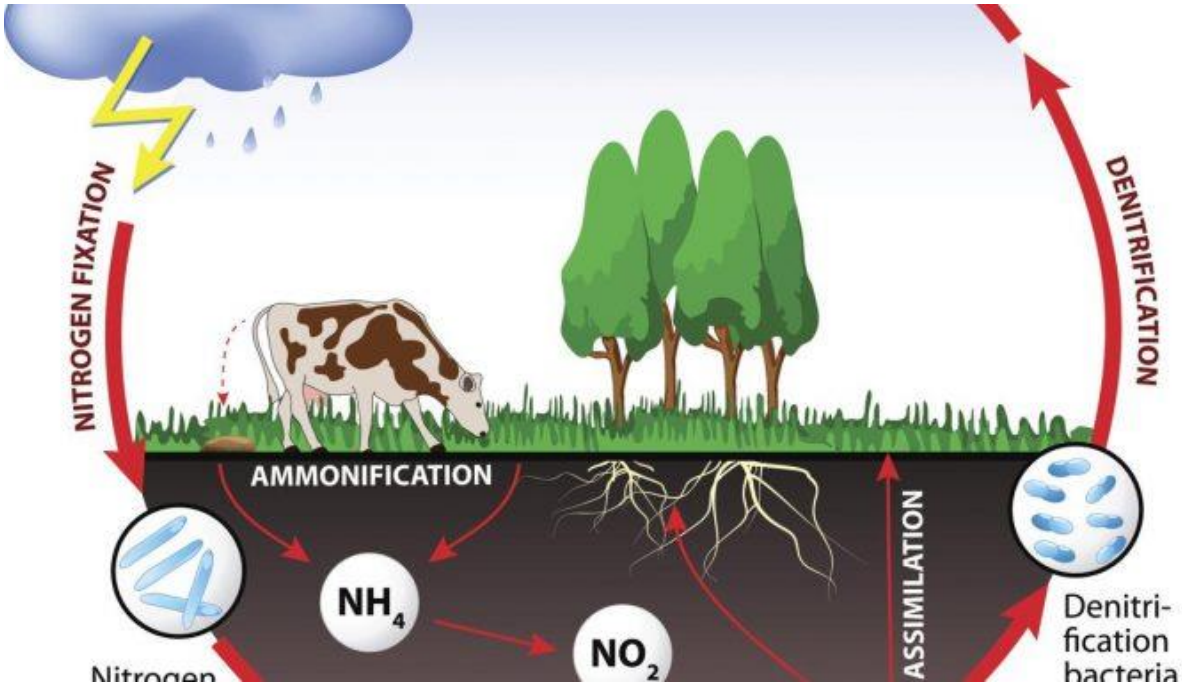
DEPARTMENT OF BIOTECHNOLOGY

UNIT – III– PLANT BIOCHEMISTRY– SBC3201

Nitrogen cycle: Steps of Nitrogen cycle



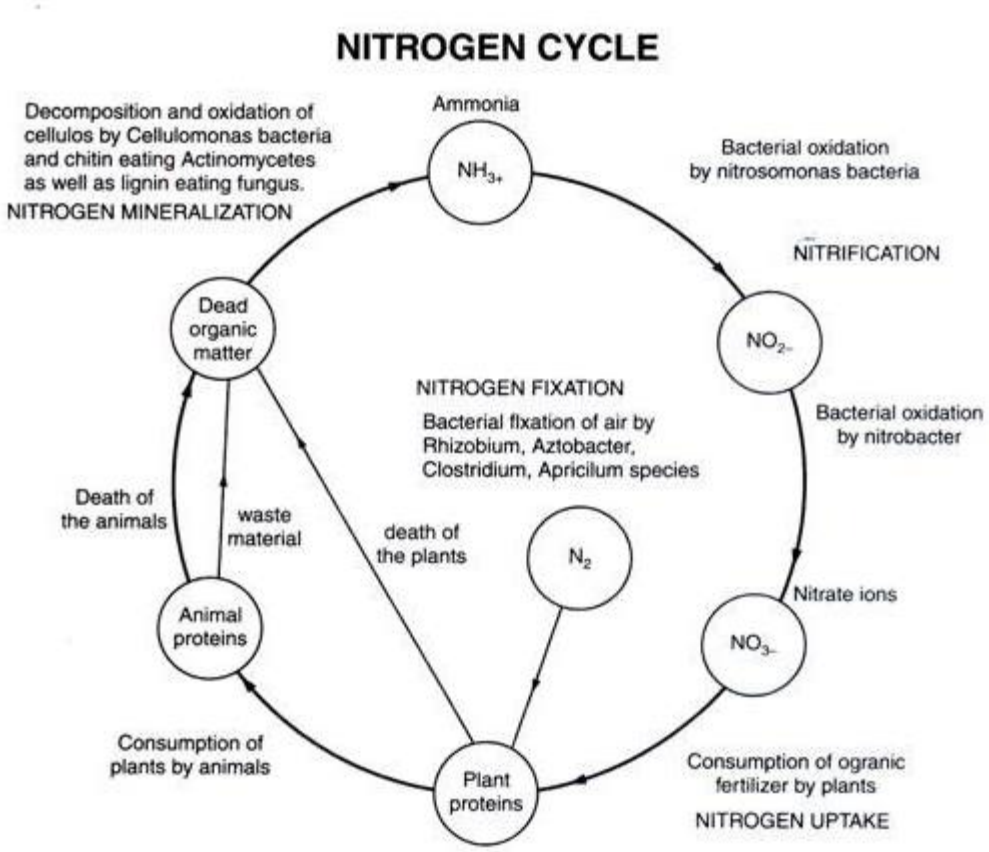
Nitrogen cycle: Steps of Nitrogen cycle

- Nitrogen undergoes a number of transformation that involves synthesis of organic compounds (amino acids, proteins, enzymes, chlorophylls, nucleic acids) as well as inorganic and volatile compounds (Ammonia, Nitrates, nitric acid). These transformation occurs simultaneously.
- N2 gas account for about 78% of air. There is very few uses of gaseous nitrogen. However, nitrogen is the most essential elements.
- Small parts atmospheric nitrogen is converted into organic nitrogen by certain free living and symbiotic nitrogen fixing microorganisms. Similarly, the nitrogen present in organic fraction of living beings is converted into ammonia (NH3) which is in turn utilized by many microorganisms or oxidized into nitrate. The nitrate may be lost into soil by leaching, may be used by plants or may be converted into atmospheric nitrogen.

The whole transformation of Nitrogen into different form is known as Nitrogen cycle

It involves following process.

1. Nitrogen fixation
2. Nitrification
3. Nitrogen assimilation/Mineralization
4. Ammonification
5. Denitrification



The nitrogen cycle

1. Nitrogen fixation:

- i. Biological nitrogen fixation
- ii. Non-biological nitrogen fixation

i. Biological Nitrogen Fixation

- Small part of atmospheric nitrogen (Nitrogen gas) is converted into biologically acceptable nitrogenous compound (NH3) by by biological organisms such as bacteria, BGA etc. and the process is called biological **nitrogen fixation.**
- Two groups of organisms are involved in this process- Symbiotic Nitrogen fixer and Non-Symbiotic Nitrogen fixer.

Symbiotic Nitrogen Fixation:

- Some bacteria living symbiotically in root nodules of legume plants can fix atmospheric nitrogen and make available for the plants. Eg. Rhizobium spp
- Some other symbiotic nitrogen fixers are:
    - Rhizobium leguminosarum: pea
    - Rhizobium phaseoli: Bean
    - Rhizobium lupine: soyabean
    - Bradyrhizobium japonicum: cow pea

Non-symbiotic Nitrogen Fixation:

- Several free living microorganisms fix atmospheric nitrogen into ammonia.
- Nitrogen fixing bacteria produces an enzyme complex known as nitrogenase that converts N2 into NH3.
- *Nitrogenase enzyme is composed of two sub-units ie. Dinitrogenase (MoFe containing protein) and dinitrogen reductase (Fe containing protein)
- The overall reaction of atmospheric nitrogen fixation by non symbiotic bacteria is absorption and reduction of atmospheric N2 into NH3.
- Many aerobic, anaerobic and facultative anaerobic bacteria as well as blue green algae can fix nitrogen non-symbiotically.
- Some Non-symbiotic (free living) nitrogen fixers:
    - Aerobic bacteria: *Azotobacter, Azomonas*, *Beijerinekia, Derxia* etc
    - Anaerobic bacteria: *Clostridium, Desulfotomaculum, Desulfovibrio* etc
    - Photosynthetic bacteria: *Rhodopseudomonas*, *Rhodomicrobium* etc
    - Blue green algae: *Nostoc, Anaebaena, Calothrix, Oscilataria* etc.

ii. Non-biological Nitrogen fixation:

- Atmospheric nitrogen react with Oxygen during lightening and thundering to produce nitrogen oxide. Then the nitrogen oxide get dissolved with rain water and fall to the ground, reacts with minerals in soil to form nitrates and ammonium salts.
- Haber's Process: Nitrogen and hydrogen reacted at high temperature and pressure to form ammonia.

$N_2 + O_2 \longrightarrow NO_2$

$NO_2 + O \longrightarrow N_2O_5$

$N_2O_5 + H_2O \longrightarrow 2HNO_3$

$2HNO_3 + CaCO_3 \longrightarrow Ca(NO_3)_2 + CO_2 + H_2O$

2. Nitrification:

- Biological formation of nitrate and nitrite from ammonia is called Nitrification.
- Nitrate is produced not only in soil by nitrifying bacteria but also in marine environment and sewage treatment plant.
- The process of nitrification is especially important in soil because the transformation of NH_4^+ ion into NO_3^- ion results in the change in charge from positive (+ve) to negative (-ve). The positively charged ion tends to bound with negatively charged clay particles in soil while negatively charged NO_3^- ion tends to freely migrate in soil water so plants can radially take up nitrate ion by roots for assimilation into organic compounds.
- Some chemoautotropic bacteria are nitrifying bacteria which acquire energy from inorganic nitrogen compound.
- There are two groups of nitrifying bacteria:
    - **i. Ammonia oxidizer:** these bacteria derive energy for cell synthesis by oxidation of ammonia and converts NH3 into nitrite.
      Examples *Nitrosomonas, Nitrococcus, Nitrospira, Nitrosolobus*

- **ii. Nitrate oxidizer**: these bacteria derive their energy by oxidation of nitrite. Example: *Nitrobacter*

3. Nitrogen assimilation and mineralization:

- Inorganic nitrogen compound present in soil after nitrogen fixation are absorbed by plants as nutrients and metabolize them for biosynthesis of aminoacids, enzymes, nucleic acids etc. the process is known as **Nitrogen assimilation.**
- When animal feeds on plants, nitrogen is deposited in the form of protein as well as converted in to other form such as urea, uric acids and excreted as faeces and urine.
- Similarly, some of the nitrogen compound undergoes **mineralization** and get deposited in the soil as ammonium salts.

4. Ammonification/ Decomposition of nitrogenous compound:

- Dead remains of plants and animals are decomposed by microorganisms (bacteria and Fungi) present in soil and convert the organic nitrogen compound into ammonia.
- Some Bacteria (*Pseudomonas, Bacillus, clostridium, Serratia*), fungi (*Alternaria*, *Aspergillus*, *Mucor*, *Penicillium*) and Actinomycetes (*Streptomyces*) can convert organic nitrogen compound into ammonia.

5. De-nitrification:

- The biological conversion of nitrite and nitrate into nitrous oxide or molecular nitrogen gas is known as de-nitrification. It is also known as **Nitrate respiration.**
- at first nitrate is reduced to nitrite which is then reduced to nitric oxide, then nitric oxide is reduced to nitrous oxide and finally to molecular nitrogen.
- De-nitrification cause loss of soil nitrogen into atmosphere. This process is known as volatilization of nitrogen.
- Denitrifying bacteria are anaerobes.
- Some examples of Denitrifying bacteria are: *Pseudomonas denitrificans, Bacillus licheniformis, Thiobacillus denitrificans, Hypomicrobium, Chromobacterium etc*

Nitrogen Fixation Types: Physical and Biological Nitrogen Fixation (With Diagram) **the two types of nitrogen fixation.**

The two types of nitrogen fixation are: (1) Physical Nitrogen Fixation and (2) Biological Nitrogen Fixation.

Apart from carbon, hydrogen and oxygen, nitrogen is the most prevalent essential macro-element in living organisms. Plants need nitrogen to build amino acids, proteins, nucleic acids, cytochromes, chlorophylls, alkaloids, phytohormones and many of the vitamins. Plants compete with microbes for limited nitrogen content available in the soil. Plants mainly absorb nitrogen in the form of nitrate (NO_3^-) or ammonium ions (NH_4^+) from the soil.

The nitrate is more abundant in well oxygenated, non-acidic soils, while ammonium is predominant in acidic or water logged soils. The other sources of available soil nitrogen may be amino acids from decaying organic matter, animal excreta (urea) and chemical fertilizers that can be absorbed directly by the plants. Nitrogen is obtained by the plants mainly from the atmosphere. It occurs as free diatomic (N_2) molecules in the air. It is highly inert gas. It cannot be used directly by the higher plants, and therefore has to be fixed. The phenomenon of conversion of free nitrogen (molecular and elemental) into nitrogenous compounds (to make it available to the plants for absorption) is called nitrogen fixation. Nitrogen fixation is carried out by physicochemical and biological means. About 10% of natural nitrogen fixation takes place by physicochemical methods and 90% by biological methods.

These are briefly discussed below:

(1) Physical Nitrogen Fixation:

(i) Natural Nitrogen Fixation:

Under the influence of lightning (i.e., electric discharge in the clouds) and thunder, N_2 and O_2 of the air react to form nitric oxide

(NO). The nitric oxides are again oxidized with oxygen to form nitrogen peroxide (NO_2).

The reactions are as follows:

$N_2 + O_2$ Lightning → Thunder 2No (Nitric Oxide); $2NO + O_2 \rightarrow 2NO_2$ Oxidation (Nitrogen peroxide)

During the rains, NO_2 combines with rain water to form nitrous acid (HNO_2) and nitric acid (HNO_3). The acids fall on the soil along with rain water and react with the alkaline radicals to form water soluble nitrates (NO_3-) and nitrites (No_2-).

$2NO_2 + H_2O \rightarrow HNO_2 + HNO_3$; HNO_3 + Ca or K salts → Ca or K nitrates

The nitrates are soluble in water and are directly absorbed by the roots of the plants.

(ii) Industrial Nitrogen Fixation:

Ammonia is produced industrially by direct combination of nitrogen with hydrogen (obtained from water) at high temperature and pressure. Later, it is converted into various kinds of fertilizers, such as urea etc.

2. Biological Nitrogen Fixation:

The conversion of atmospheric nitrogen into the nitrogenous compounds through the agency of living organisms is called biological nitrogen fixation. The process is carried out by two main types of microorganism: those which live in close symbiotic association with other plants and those which are “free living” or non-symbiotic.

Biological nitrogen fixation (BNF) is the process whereby atmospheric nitrogen is reduced to ammonia in the presence of nitrogenize. Nitrogenize is a biological catalyst found naturally only in certain microorganisms such as the symbiotic Rhizobium and Frankia, or the free-living Azospirillum and Azotobacter and BGA.

Details of biological nitrogen fixation follow.

Nearly 80% of Earths atmosphere contains nitrogen in the form of a highly inert di-nitrogen (N = N) which most plants cannot utilize as such. The atmospheric di-nitrogen (N_2) consists of two nitrogen atoms linked by a triple-covalent bond. About 225 kcal of energy is required to break this triple bond which is difficult to achieve.
The phenomenon of reduction of inert gaseous di-nitrogen (N_2) into ammonia (NH_3) through the agency of some microorganisms so that it can be made available to the plants is called as biological nitrogen fixation or diazotrophy.

Nitrogen Fixers:

Among the earth's organisms, only some prokaryotes like bacteria and cyanobacteria can fix atmosphere nitrogen. They are called nitrogen fixers or diazotrophs. They fix about 95% of the total global nitrogen fixed annually (-200 million matric tones) by natural process.

Diazotrophs may be asymbiotic (free living) or symbiotic such as given below:

(i) Free Living Nitrogen Fixing Bacteria:

Azotobacter, Beijerinckia (bothaerobic) and Clostridium (anaerobic) are saprophytic bacteria that perform nitrogen fixation. Desulphovibrio is chemotrophic nitrogen fixing bacterium. Rhodopseudomonas, Rhodospirillum and Chromatium are nitrogen fixing photoautotrophic bacteria. These bacteria add up to 10-25 kg, of nitrogen/ha/annum.

(ii) Free living Nitrogen Fixing Cyanobacteria:

Many free living blue-green algae (now called cyanobacteria) perform nitrogen fixation, e.g., Anabaena, Nustoc, Aulosira, Cylmdrospermum, Trichodesmium. These are also important ecologically as they live in water-logged sods where denitrifing bacteria can be active. Aulosira fertilissima is the most active nitrogen fixer in Rice fields, while Cylindrospermum is active in sugarcane and maize fields. They add 20-30 kg Nitrogen/ha/annum.

(iii) Symbiotic Nitrogen Fixing Cyanobacteria:

Anabaena and Nostoc species are common symbionts in lichens, Anthoceros, Azolla and cycad roots. Azolla pinnata (a water fern) has Anabaena azollae in its fronds. It is often inoculated to Rice fields for nitrogen fixation.

(iv) Symbiotic Nitrogen Fixing Bacteria:

Rhizobium is aerobic, gram negative nitrogen fixing bacterial symbionts of Papilionaceous roots. Sesbania rostrata has Rhizobium in root nodules and Aerorhizobium in stem nodules. Frankia is symbiont in root nodules of many non-leguminous plants like Casuarina and Alnus.

Xanthomonas and Mycobacterium occur as symbiont in the leaves of some members of the families Rubiaceae and Myrsinaceae (e.g., Ardisia). Several species of Rhizobium live in the soil but are unable to fix nitrogen by themselves. They do so only as symbionts in the association of roots of legumes.

Symbiotic Nitrogen Fixation:

Both Rhizobium sp. and Frankia are free living in soil, but only as symbionts, can fix atmospheric di-nitrogen.

The symbiotic nitrogen fixation can be discussed under following steps:

(i) Nodule formation (Fig. 5.1):

It involves multiple interactions between free-living soil Rizobium and roots of the host plant. The important stages involved in nodule formation are as follows-Host Specificity: A variety of microorganisms exist in the rhizosphere (i.e. immediate vicinity of roots) of host roots.

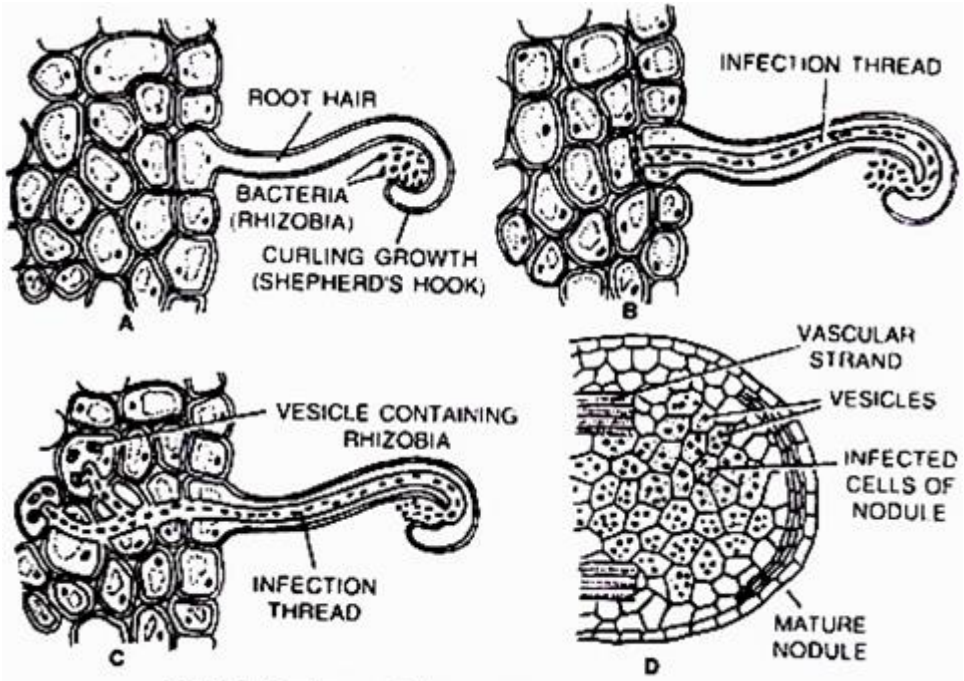


Fig. 5.1 Development of root nodule (nodulation) in legume.

The roots of young leguminous plants secrete a group of chemical attractants like flavonoids and betaines. In response to these chemical attractants specific rhizobial Tells migrate towards the root hairs and produce nod (nodulation) factors. The nod factors found on bacterial surface bind to the lectin proteins present on the surface of root hairs. This lectinnod factor interaction induces growth and curling of root hairs around Rhizobia.

At these regions wall degrades in response to node-factors and Rhizobia enter the root hair invagination of plasma membrane called infection thread. The infection thread filled with dividing Rhizobia elongate through the root hair and later branched to reach different cortical cells.

The Rhizobia are released into the cortical cells either single or in groups enclosed by a membrane. The Rhizobia stop dividing, loose cell wall and become nitrogen fixing cells as led bacteroids .The membrane surrounding the bacteroids is called peribacteroid membrane. The infected cortical cells divide to form nodule (Fig. 5.2).

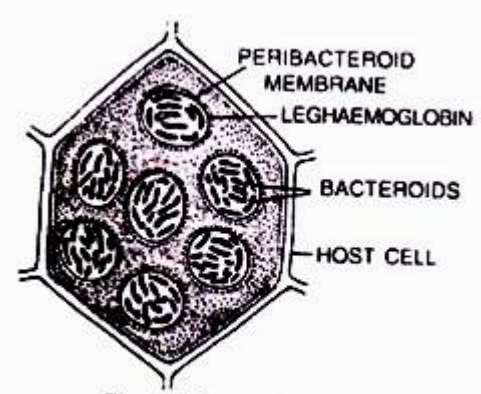


Fig. 5.2 Bacteroids in an infected cortical cell.

(ii) Mechanism of nitrogen fixation (Fig 5.3):

The nodule serves as site for N_2 fixation. It contains all the necessary bio-chemicals such as the enzyme complex called nitrogenase and leghaemoglobin (leguminous haemoglobin). The nitrogenase has 2 components i.e. Mo-Fe protein (molybdoferredoxin) and Fe-protein (azoferredoxin).The nitrogenase catalyzes the conversion of atmosphere di-nitrogen (N_2) to $2NH_3$. The ammonia is the first stable product of nitrogen fixation.

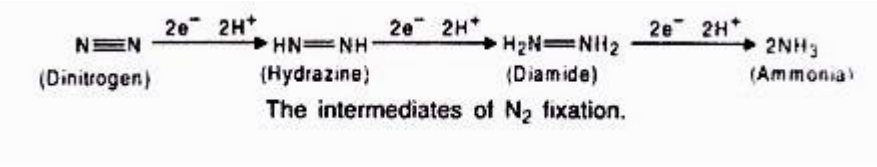
The overall equation is:

$$N_2 + 8e^- + 8H^+ + 16\ ATP \xrightarrow[\text{Nitrogenase}]{Mg^{2+}} 2NH_3 + H_2 + 16ADP + 16\ Pi$$

The nitrogenase is extremely sensitive to oxygen. To protect these enzymes, nodule contains an oxygen scavenger called leghaemoglobin (Lb), which is a reddish-pink pigment. There are two views about location of leghaemoglobin that is either located outside the peribacteroid membrane or located in between bacteroids.

During nitrogen fixation, the free di-nitrogen first bound to MoFe protein and is not released until completely reduced to ammonia. The reduction of di-nitrogen is a stepwise reaction in which many intermediates are formed to form ammonia (NH_3) which is protonated at physiological pH to form NH4+. In this process ferredoxin serves as an electron donor to Fe-protein (nitrogenase reductase) which in turn hydrolyzes ATP and reduce MoFe protein, the MoFe protein in Turn reduce the substrate N_2. The electrons

and ATP are provided by photosynthesis and respiration of the host cells.



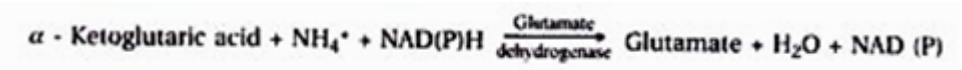
The intermediates of N_2 fixation.

Assimilation of Ammonia:

The ammonia produced by nitrogenase is immediately protonated to form ammonium ion (NH_4+). As $NH4^+$ is toxic to plants, it is rapidly used near the site of generation to synthesize amino acids. Amino acids synthesis takes place by three methods: reductive animation, catalytic amination and transamination.

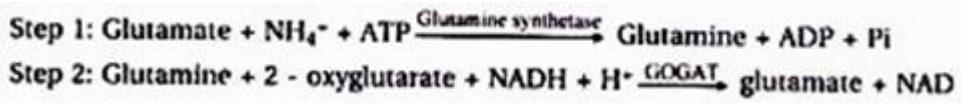
(i) Reductive amination:

In this process, glumate dehydrogenase (GDH) catalyzes the synthesis of glutamic acid.



(ii) Catalytic amidation:

It is a two step process catalyzed by glutamine synthetase (GS) and glutamate synthetase (glutamine – 2-oxyglutarate aminotransferase, or GOGAT).



Out of the two glutamates produced one returns to GS while the other is exported to the plant.

(iii) Transamination:

Glutamate or glutamic acid is the main amino acid from which other amino acids are derived through transamination. The enzyme aminotransferases (= transaminases) catalyze all such reactions. Transamination involves transfer of amino group from one amino acid to the keto group of keto acid.

Glutamate (amino donor) + Oxaloacetate (amino acceptor) → Aspartate (amino acid) + 2 oxyglutarate

In nitrogen fixing plants, the fixed nitrogen is exported in the form of amides (asparagines and glutamine) and Ureides (allantoin,

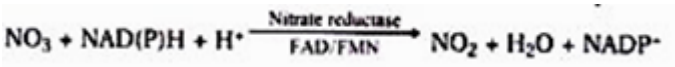
allantoic acid and citrulline), from the nodules to other plant parts via xylem. Amides are formed from two amino acids, namely glutamic acid and aspartic acid, by replacing – OH part by another NH_2– radicle. Thus, amides contain more nitrogen than amino acids and are structural part of most proteins.

Nitrate Assimilation:

Nitrate cannot be utilized by plants as such. It is first reduced to ammonia before being incorporated into organic compounds. Reduction of nitrate occurs in two steps:

1. Reduction of nitrate to nitrite:

It is carried out by an inducible enzyme, nitrate reductase. The enzyme is a molybdoflavoprotein. It requires a reduced coenzyme NADH or NADPH for its activity which is brought in contact with nitrate by FAD or FMN.



2. Reduction of nitrate:

It is carried out by the enzyme nitrite reductase. The enzyme is a metalloflavoprotein which contains copper and iron. It occurs inside chloroplast in leaf cells and leucoplast of other cells. Nitrite reductase require reducing power. It is NADPH and NADH (NADPH in illuminated cells).

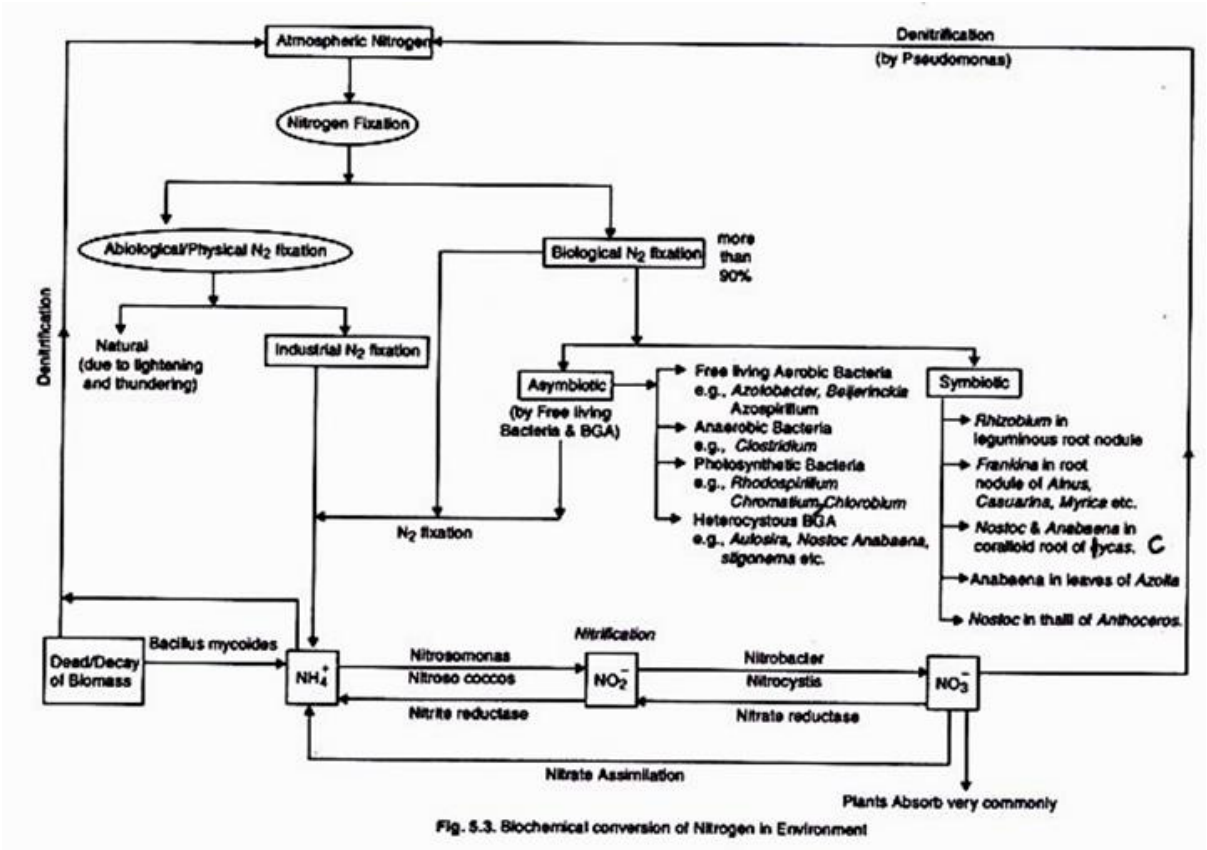


Fig. 5.3. Biochemical conversion of Nitrogen in Environment

Reduction process also require ferredoxin which occurs in green tissues of higher plants. It is presumed that in higher plants either nitrite is trans-located to leaf cells or some other electron donor (like FAD) operates in un-illuminated cells. The product of nitrite reduction in ammonia.

$$2NO_2^- + 7NAD(P)H + 7H^+ \xrightarrow[\text{Ferredoxin}]{\text{Nitrite reductase}} 2NH_3 + 4H_2O + 7NAD(P)^+$$

Ammonia thus produced combines with organic acids to produce amino acids. Amino acids form protein by the process of translation.

Phytohormones: Types and physiological effects in plant growth and development

Phytohormones: Types and physiological effects in plant growth and development

What is Plant hormone?

- Plant hormones are also termed as phytohormones (named by Thieman), growth factors, growth regulators, growth substances etc.
- Phytohormone is an organic substance, naturally produced in higher plants that regulate plant physiological process such as affecting growth and other functions remote from its place of production and active in very minute amounts.
- They can be either natural or synthetic, stimulatory or inhibitory in nature.
- They act at a distance from the place where they are formed.
- Three types of phytohormones are mostly recognized. They are:
    - Auxin
    - Gibberellin
    - Cytokinin

1. Auxin:

- An auxin is an organic compound responsible for promoting the growth of plants along the longitudinal axis when applied in low concentrations to shoots of the plants.
- Auxin is specifically concerned with cell enlargement or the growth of the shoots.
- Auxin is identical to Indole 3-Acetic Acid ($C_{10}H_9O_2N$, IAA), i.e. natural true auxin.
- The precursor of Indole 3-Acetic Acid is tryptophan and zinc play a role in its biosynthesis.
- Auxin exhibits polar movement i.e.
- Basipetal movement (from apex to base) in case of shoots.
- Acropetal movement (from root tip to shoot) in case of roots.
- Bioassay test: Bioassay is termed as the functional test of substance in living plants.
- The common bioassay tests of auxin are Avena coleoptile test and root growth inhibition test.

What are the physiological roles of auxin in plants?

- Besides the cell enlargement and growth, auxin (both natural and synthetic) are responsible for various other growth processes. They are:
- **Cell elongation:**

- The cell elongation occurs only in the presence of auxin and the rate of elongation is directly proportional to the amount of auxin supplied, given no other factors are limiting.
- However, relatively high concentrations of auxin show inhibitory effects on this phase of growth.
- Auxin promotes the elongation of roots at its low concentrations, the growth of roots is retarded at higher concentrations.
- Flowers need higher concentration of auxin for their growth.
- Auxin also induces the elongation of coleoptiles and stems by cell enlargement.
- Auxins are responsible for the elongation of petiole, mid rib and major lateral veins of the leaves.
- Hence, adenine aids in enlargement in detached leaves of radish and pea. Similarly, coumarin has been shown to promote expansion of leaves in some plants.

- **Cambial activity:**
  - During the spring season, the trees manifest growth by developing buds that later on open and elongation of young stems take place.
  - Auxin activates this resumed growth by cambial cells
  - The growth moves basipetally in the stems from developing buds.
- **Callus formation and galls:**
  - The auxins activate cell division.
  - When 1% IAA in lanolin paste is applied to a de-bladed petiole of a bean plant, prolific division of parenchyma cells occurs.
  - A swelling or callus tissue is formed at the point of application of auxin.
  - The amount of callus tissue formed is directly proportional to the concentration of IAA applied.
- **Apical dominance:**
  - Apical dominance is the major function of auxin.
  - The growth of lateral buds is suppressed until apical bud is present in the plants.
  - This inhibitory effect of terminal bud upon the growth of lateral buds is termed as apical dominance.
  - Skoog and Thimann (1934) first reported the relation of apical dominance
    with the auxin supply.
  - When agar block containing auxin b or IAA was kept on the decapitated shoot of broad bean (*Vicia faba*), the lateral buds, as might be expected, resulted in the usual suppression of growth.
  - But when the same decapitated shoot was re-headed with an agar block containing no auxin, these lateral buds resumed growth.
  - When NAA was used as auxin in field-grown tobacco plants, similar results were obtained.
  - Evidence of apical dominance has been practically used in solving the potato storage problem.
  - Potatoes, stored for some time, sprout and become sweet in taste, causing the grower to lose financially as its consumers hate the sweet taste.

- But by inhibiting the growth of buds or ‘eyes’ by spraying potatoes with auxins such as indole butyric acid (IBA) and NAA, sprouting (or in other words, prolonging dormancy) can stop sprouting; the effect lasts for as long as 3 years.

- **Rooting of stem cuttings (Formation of adventitious roots):**
  - It is a common observation that when the lower end is dipped in an acceptable rooting medium, the appearance of buds on a cutting promotes the growth of roots.
  - In accelerating root formation, developing buds are efficient.
  - The initiation of roots on the cuttings are often favoured by young leaves.
  - These findings contributed to the idea that the auxins synthesized in the buds and young leaves favour the root formation and are later translocated to the basal part of the cut.
  - IAA, NAA, 2,4-D, naphthalene acetamide (NAd) etc are the auxins most widely used for this function.
  - Auxin-induced rooting is also of considerable horticultural benefit as it allows cuttings to propagate those plants.
- **Delay (or inhibition) of abscission of leaves:**
  - By adding auxins on the surface of the lamina or on the cut surface of a debladed petiole, abscission of the leaves may be delayed or hindered.
  - Laibach (1933), who demonstrated that the extract of orchid pollinia is capable of preventing leaf dropping, first noted the regulating actions of auxins on abscission.
  - Since then, sufficient work in this direction has been carried out.
  - The delaying effect of IAA on the abscission of different plant organs has been shown conclusively by Addicott and Lynch (1955).
  - As for the abscission process, it has been proposed that the basipetal migration of a hormone from the blade to the base of the petiole retards the leaf drop.
  - Leaf blade removal removes the hormone supply to the abscission zone and thus causes the drop of the leaf.
- **Flowering:**
  - Auxins play role in modifying flowering by following ways:
  - Producing early flowering
  - Inducing flowering
  - Preventing or delaying flowering
- **Fruiting:**
  - Auxins play significant role in fruiting by altering it in one of the following ways :
  - **Fruit setting:**
  - The changes in the ovary leading to the development of the fruit is termed as fruit set.
  - These changes are generally induced after pollination and fertilization.
  - The development of fruit without fertilization is termed as parthenocarpy.
  - It is a common characteristics in plants and hence occurs frequently.
  - The parthenocarpy can be induced artificially by the aid of auxin.

- For example, Yasuda (1934) demonstrated it by application of pollen extracts to cucumber flowers.
- It was also observed that ovaries of many plants (orange, lemon, grape, banana, tomato etc.) could be induced to develop into seedless fruits by application of IAA in lanolin paste to their stigmas.
- The various other auxins used for parthenocarpy are IPA, IBA, α-NAA, phenoxyacetic acid (POA), α- naphthoxyacetic acid (NOA) etc.
- **Fruit thinning:**
- The trees, in many cases, bear a large number of fruits.
- It leads to the inability of the trees to grow an average number of new flower buds.
- Therefore, such trees must grow fruit either in alternate years (alternate bearing) or if yearly, the number of fruits is significantly reduced.
- Clearly, these trees need thinning.
- For the first time, fruit thinning was achieved in apples when naphthalene acetic acid added to flowers failed to set the fruits and actually caused a decrease in the set of fruits.
- It is interesting to note that the only effective auxin that induces fruit thinning seems to be naphthalene acetic acid.
- However, a-2,4,5-trichlorophenoxyacetic acid for thinning of pears and p-chlorophenoxyacetic acid for thinning of grapes are other examples of auxins used for fruit thinning.
- **Control of premature fruit dropping:**
- The development of an abscission sheet causes the falling of unripe fruits in many fruit trees causes significant losses in yield to the gardeners.
- In several cases, such as apples, the problem has now been successfully overcome by the application of auxins.
- Auxins prevent the formation of the abscission layer and thus check the drop of the fruits before harvesting.
- With 2,4-D and 2,4,5-trichlorophenoxyacetic acid as auxins, regulation has also been induced in citrus fruits (like oranges and lemons).
- **Improving the quality of fruits:**
- The different processes such as colouring, softening, sweetening and ripening are all involved in improving the fruit ‘s quality.
- In apples, where the use of 2,4, 5-trichlorophenoxyacetic acid has significantly increased red pigments, the auxin effects on fruit colouration are most noticeable.
- 2,4-D accelerated the ripening process when added to bananas as the auxin stimulates the conversion of starch into sugars.
- Sugar accumulation has been reported in sugarcane by injecting 2,4-D, IBA or maleic hydrazide.

- **Increase in respiration:**
  - Auxins enhances the respiration process. It was first identified by James Bonner in 1953.
  - A direct relation between growth due to auxin treatment and rate of respiration has been found i.e., greater the growth, higher is the respiration.
  - Auxins are used to control the growth of weeds in the crop fields.

        - 2,4-D is sprayed for the weeds in the crop fields that acts as weed killer.
        - Graminaceous weeds are destroyed by 2,4-dichloropropionic acid.
- **Increased resistance to frost damage:**
    - When parsnip is treated by 2,4,5-T, the tops resist damage by frost.
    - In apricot fruits, the application of 2,4,5-T before the onset of frost caused less damage than the untreated fruits.
- **Great weapon of war:**
    - When auxins are applied in higher concentrations on enemy crop fields by means of air, it causes devastation of land and form the basis for biological warfare.

2. Gibberellins:

- E. Kurosawa, first discovered gibberellin from a fungus called *Gibberella fujikoroi* in the year 1926.
- A *gibberellin* is abbreviated as GA, for gibberellic acid.
- Gibberellin may be referred as a compound which is active in gibberellin bioassays and possesses a gibbane ring skeleton.
- However, there are other compounds (like kaurene) that are active in some of the assays but lack a gibbane ring. Such compounds have been termed *gibberellin-like* rather than gibberellins.
- Brian isolated pure sample of a single gibberellin and termed as gibberellic acid.
- The structure for gibberellic acid was given by Cross et al in 1961.
- More than 100 types of gibberellin are known, among them GA_3 is most common.
- Gas are common in all groups of plants, however it acts as a flowering hormone in angiosperms only.
- All gibberellin possess gibbane ring. Gibbane ring consists of 4 isoprene units (hence, 2 terpenes, di-terpenes).
- The 5 carbon compound isopentenyl pyrophosphate is the precursor of gibberellin.
- Bioassay test: Gibberellins are synthesized via the mevalonic acid (MVA) pathway.
- The biosynthesis of GA3 from MVA takes place by 18 or more steps or intermediates and about 15 associated compounds.

What are major Physiological effects of Gibberellin in plants?

- **Genetic dwarfism:**
    - In some plants, the mutation of a single gene causes dwarfism.
    - Such individuals are termed as ‘single gene dwarfs’.
    - In these plants dwarfism is due to shortening of internodes rather than reduction in number of internodes.
    - The use of gibberellins on such dwarfs causes them to elongate to the point of being indistinguishable from common tall plants.
    - Hence, gibberellin A3 treatment has been used to overcome genetic dwarfism successfully in many single gene dwarf mutants like *Pisum sativum, Vicia faba and Phaseolus multiflorus.*
    - Gibberellin also induces leaf expansion.
- **Bolting and flowering:**
    - Rosette plants are marked by the prolific growth of leaves and the delayed growth of internodes.

- But there is striking elongation in the internode before the reproductive process, so that the plant reaches 5 to 6 times the initial height.
- The treatment of these 'rosette' plants with gibberellins stimulates bolting (or shoot elongation) and flowering under conditions that would normally preserve the rosette shape.
- It is also possible to distinguish shoot elongation from flowering by controlling the amount of gibberellin applied.
- The plant can bolt but not flower with low gibberellin dosages.
- GA_3 hastens the flowering and flower yield in many plants such as *Coriandrum sativum* (coriander).
- Gibberellin controls flowering in long day plants.

- **Light inhibited stem growth:**
  - The dark-grown plants showed better stem growth in comparison to light grown plants.
  - This inhibitory effect of light on stem elongation could be reversed by the use of gibberellins in plants as such *Pisum sativum.*
  - This clearly indicates that the gibberellin is the limiting factor in stem elongation.
- **Parthenocarpy:**
  - Gibberellins induce parthenocarpy more efficiently than auxins.
  - It has been found in plants such as *Cucumis sativa (*cucumber), *Zepyranthes* sp., *Solanum melongena* (brinjal).
- **Breaking dormancy of seeds:**
  - In the light sensitive seeds (lettuce, tobacco), the germination is retarded in dark.
  - The application of GA_3 allows the germination of seeds in dark as well.
- **Breaking dormancy of buds:**
  - Because of very low temperature, the buds produced in winter stays dormant till the next spring in temperate areas.
  - Gibberellin treatment overcomes the dormancy in such cases and replaces the light requirement for breaking dormancy.
  - It breaks dormancy in potato tubers as well.
- **Role in abscission:**
  - The abscission has been enhanced in explants of bean and *Coleus* by the GA_3 treatments.
- **Stimulation of enzyme activity in cereal endosperm:**
  - It was demonstrated that the exogenous application of gibberellins stimulated amylase activity in isolated barley endosperm.
  - It was also found that the treatment of isolated aleurone layer of endosperm with GA could release enzymes, amylase and proteinase.
- **Sex expression:**
  - Gibberellins show the capability to alter the sex of the flowers.
  - It promotes the production of male flowers in cucurbitis, *Cannabis* etc.
  - Also, the antheridia have been induced to form in many fern gametophyte
- **Juvenility:**
  - Most of the plants manifests two different stages of growth i.e. a juvenile stage and an adult stage.

- The application of gibberellin helps to determine if a specific part of plant is juvenile or not.

3. Cytokinins:

- Cytokinins are alos named as kinetins because of their absolute power to enhance cell division in the presence of an auxin.
- First naturally occurring cytokinin was recognized from young maize grain by Letham and termed as zeatin.
- Fox (1969) has defined cytokinins as chemicals composed of one hydrophilic adenine group of high specificity and one lipophilic group without specificity.
- *Chemically, kinetin* (C10H9ON5) is 6-furfurylaminopurine.
- Cytokinins occur in higher plants, diatoms, red and brown algae, mosses.
- These occur widely in embryo sac, roots during seedling stage, flowers, developing fruits, cambial tissue and endosperm.
- The richest source of kinins are fruits and endosperm.
- Bioassay test: Callus pith cell division, chlorophyll retention test, soybean and radish cotyledon cell division are the main bioassay tests.

What are Physiological roles of cytokinin in plants?:

- **Cell division:**
  - In addition to auxins, the kinins are required in right ratio of concentrations for the enormous growth response .
  - When mixture of auxin and cytokinin is added to unspecialized cells, their differentiation begins.
  - A high cytokinin to auxin ratio results the formation of shoots, buds and leaves while a low cytokinin to auxin ratio causes root formation.
  - This invitro culture methods allows the rapid production of large number of plants in a small space.
- **Cell elongation:**
  - Kinetin also enhances cell elongation.
  - It has been demonstrated in tobacco pith cultures, tobacco roots and bean leaf tissues.
- **Root growth:**
  - Kinetin is responsible for both the stimulation as well as inhibition of root development.
  - When kinetin was applied along with IAA, the root initiation and development in stem callus cultures was stimulated.
  - In lupin seedlings, Kinetins induced increase in dry weight and elongation of the roots.
- **Shoot growth:**
  - When the balance of IAA and kinetin is maintained, the callus tissue of tobacco can be kept in an undifferentiated state for a long time.
  - When the amount of kinetin is increased, the development of leafy shoots begins.
- **Organogenesis:**
  - Organogenesis is resulted by cytokinins in several tissue cultures.
  - By changing the relative concentrations of kinetins and auxins, the tobacco pith callus can be directed to develop either buds or roots.

- High kinetin and low auxin contents causes the production of buds.
- The roots appear in pith in reverse condition, i.e. high auxin and low kinetin contents.
- In leaf segments of various plants such as *Saintpaulia ionantha, Bryophyllum sp and Begonia* sp., the kinins stimulate the production of buds.
- In addition to the root and shoot differentiation, the cytokinins also bring about other morphogenetic responses.
- These are :
  (a) maturation of proplastids into plastids
  (*b*) differentiation of tracheids
  (*c*) induction of parthenocarpy
  (*d*) induction of flowering

- **Counteraction of apical dominance:**
  - Cytokinins are powerful promoters of lateral bud growth.
  - When the culture medium consists of IAA, the growth of lateral buds is inhibited, but the addition of kinetin along with IAA stimulates the growth of lateral buds.
- **Breaking dormancy of seeds:**
  - Cytokinins show effective role in breaking seed dormancy in lettuce, tobacco, white clover and carpet grass.
  - In such cases, the site of cytokinin action is cotyledon.
  - The seeds of parasites such as Striga asiatica need the host plant for germination. But when treated with kinetin, the seeds germinate even in the absence of their host.
- **Delay of senescence (Richmond-Lang effect):**
  - The ageing of leaves along with the loss of chlorophyll and the breakdown of proteins is termed as senescence.
  - Richmond and Lang demonstrated that the senescence in the detached leaves of Xanthium could be postponed for several days by kinetin treatment.
  - This effect of kinetin in retarding senescence is termed as Richmond-Lang effect.
- **Role in abscission:**
  - Depending on the site of application, cytokinins can either accelerate or retard the process of abscission in leaf petioles.
  - It is the common property of cytokinin.
- **Effects on cotyledons:**
  - Cytokinins enhances cellular division and expansion in cotyledons.
  - Cytokinins increase the concentration of sugars in cells resulting in endosmosis that causes the expansion of cytokinin treated cells in cotyledons.

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SCHOOL OF BIO AND CHEMICAL ENGINEERING

DEPARTMENT OF BIOTECHNOLOGY

UNIT – IV– PLANT BIOCHEMISTRY– SBC3201

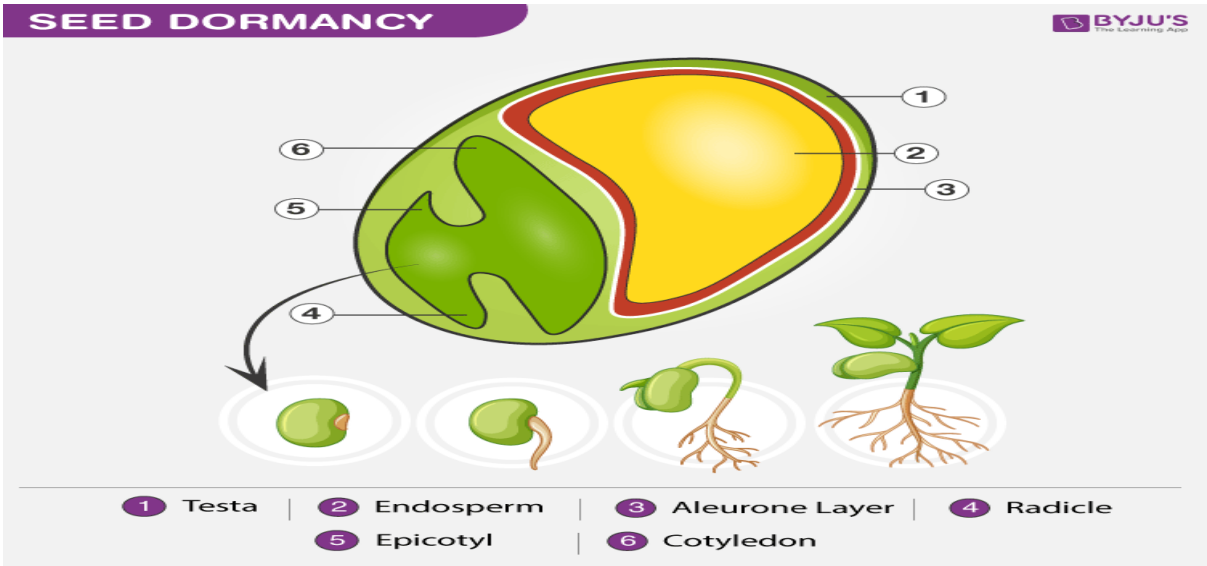
SEED DORMANCY

Seed dormancy can be defined as the state or a condition in which seeds are prevented from germinating even under the favourable environmental conditions for germination including, temperature, water, light, gas, seed coats, and other mechanical restrictions.

The main reason behind these conditions is that they require a period of rest before being capable of germination. These conditions may vary from days to months and even years. These conditions are the combination of light, water, heat, gases, seed coats and hormone structures.

Also Refer: Seed Germination

Reasons or Causes of the Seed Dormancy



There are certain major causes for the seed dormancy. Listed below are the few reasons for the seed dormancy.

- Light
- Temperature
- Hard Seed Coat
- Period after ripening
- Germination inhibitors
- Immaturity of the seed embryo
- Impermeability of seed coat to water
- Impermeability of seed coat to oxygen
- Mechanically resistant seed coat
- Presence of high concentrate solutes

Types of Seed Dormancy

The seed dormancy is of following types:

Innate dormancy

It is the condition of seeds which is incapable of germination even if conditions suitable for seedling growth are supplied. This inability to germinate may be due in certain species to the embryo being immature at the time of dispersal.

Enforced dormancy

It is the condition of seeds which is incapable of germination due to an environmental restraint which includes, an adequate amount of moisture, oxygen, light and a suitable temperature.

Induced dormancy

This type of seed dormancy occurs when the seed has imbibed water, but has been placed under extremely unfavourable conditions for germination. Finally, seed fails to germinate even under more favourable conditions.

Methods of Breaking Seed Dormancy

The different methods of breaking dormancy are mentioned below:

The natural breaking of Seed Dormancy

Nature of dormancy stops when the embryo gets appropriate environment such as adaptive moisture and temperature. The seed coat that exists in many species becomes permeable due to the rupturing of smoothing action of natural agents like microorganism, temperature, and abrasion by the digestive tract of birds and animals that feed on these seeds. Other natural methods include:

- Completion of the over-ripening period.
- Leaching of inhibitors present in the seed coat.
- Inactivation of inhibitors by the supply of cold, heat, and light.
- Leaching of the excess and highly concentrated solutes from the seeds.
- Production of growth hormones which can neutralize the effect of inhibitors.

Artificial Overcoming of Seed Dormancy

Some of the artificial methods used for breaking seed dormancy are listed below:

- Action with hot water for termination of waxes, surface inhibitors, etc.
- Rupturing of seed coats by filing, chipping, or threshing through machines.
- Exposure to heat, cold or light, depending upon the type of seed dormancy.
- By applying Hydraulic pressure for 5 to 20 minutes in order to weaken the tough seed coats.
- Seed coats are treated with concentrated sulphuric acid for removing all traces of the mineral acid.

Treatment to break dormancy in seeds

There are separate treatments to overcome dormancy, and they are further divided into the following groups:

Seed coat treatment

These treatments make a hard seed coat permeable to water or gases either by softening or cracking. This process is called scarification. The treatment can be either chemical or physical in nature.

Embryo treatments

Stratification: The incubation of seeds at an appropriate low temperature over a moist layer before transferring to a temperature suitable for germination.

High-temperature treatment: Incubation at 40-50 °C for a few hours to a few days may have an effect in overcoming dormancy in some species. For instance, rice seeds treated with hot water at 40°C for at least 4 hours.

Chemical treatments

Plant growth regulators or other chemicals can be used in induced germination growth regulators.

Importance of Seed Dormancy

1. It follows the storage of seeds for later use by animals and man.
2. It helps in the dispersal of the seeds through the unfavourable environment.
3. Dormancy induced by the inhibitors present in the seed coats is highly useful to desert plants.
4. Allows the seeds to continue to be in suspended animation without any harm during cold or high summer temperature and even under drought conditions.
5. Dormancy helps seeds to remain alive in the soil for several years and provides a continuous source of new plants, even when all the mature plants of the area have died down due to natural disasters.

Phytochrome: Discovery, Structure and Mode of Action

1. Discovery of Phytochrome 2. Structure and Biosynthesis of Phytochrome 3. Mode of Action.

Discovery of Phytochrome:

Phytochrome is a blue protein pigment responsible for the perception of light in photo-physiological processes. It is possibly the only photoreceptor in photoperiodism and the flowering process.

The discovery of phytochrome is closely associated with studies on flowering. However, many other light controlled plant responses other than photosynthesis, collectively called photo-morphogenesis, are the effects of phytochrome action.

In 1932, Beltsville research group of the USDA headed by Borthwick and Hendricks showed that red light (630 to 680 nm) elicits the germination of lettuce seeds, whereas far-red light (710 to 740 nm) inhibits the process.

It was further observed that when lettuce seeds were exposed to alternating red and far-red light, almost all seeds that received red light as the final treatment germinated, whereas the seeds receiving far-red light as the final treatment did not germinate.

The phytochrome involvement in the flowering process was envisioned when in 1952. Borthwick. Hendricks and Parker demonstrated that red light inhibition of flowering in Xanthium could be reversed by a subsequent far-red light treatment.

The action spectra for inhibition and promotion of flowering shows that the red light near 660 nm and far-red light near 730 nm respectively, are maximally effective. When the plant is subjected to several consecutive irradiations with red and far-red in sequence, the flowering response is determined by the wavelength of the final exposure (Table 1 4.2).

Table 14.2 : Effect of Successive Red (R)/Far-red (FR) Cycles Given in the Middle of an Inductive Dark Period on Flower Initiation in the SDP *Xanthium strumarium*

Treatment near middle of 12-hour dark period on three successive days*	Mean stage of floral development 10 days after plants were returned to long-day conditions**
Dark control	6.0
R	0.0
R–FR	5.6
R–FR–R	0.0
R–FR–R–FR	4.2
R–FR–R–FR–R	0.0
R–FR–R–FR–R–FR	2.4

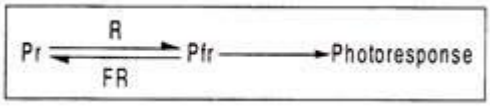
* R = 2 min red light, FR = 2 min far-red light

** Arbitrary index of floral primordium development scoring various stages from 0 (i.e., completely vegetative) to 7.

(from Downs, 1956)

From these observations, Borthwick and his associates concluded that phytochrome, the photoreceptor, exists as two inter-convertible forms, one absorbing red radiation (Pr or P_{660}), the other with an absorption maximum in the far-red region of the spectrum (Pfr or P_{730}). The Pr form of pigment is converted by red light (R) to the Pfr form, conversely, far-red light (PR) changes the Pfr form of pigment to Pr. The effect of natural white light is like red light (R). Pfr form of phytochrome, produced by exposure to R, is thought to be the physiologically active form since relatively small amount of Pfr brings about a response.

A simple model of phytochrome action can be represented as follows:



In 1959, Butler and his associates first extracted phytochrome from etiolated oat coleoptiles. It occurs as a chromo-protein in which the chromophore is a linear tetrapyrrole similar to C-phycocyanin.

Phytochrome is widely distributed in the plant kingdom. Although green leaves are the organs, which perceive day light most effectively, their phytochromecontent is very low. For this reason, most studies with phytochrome have used etiolated seedlings from which phytochrome has been obtained in highly purified form.

The absorption spectra of the two forms of phytochrome, i.e., P660 and P730 overlap considerably (Fig. 14.1). The overlap is the reason why total photochemical conversion is not possible when irradiated with either red or far-red. When we irradiate the system with red light (660 nm), about 75% of the total phytochrome can be present as P730 (Pfr) at photochemical equilibrium.

Under irradiation with far-red (730 nm), the proportion of Pfr to total phytochrome (Pfr/P) is usually 3% at photo-stationary state.

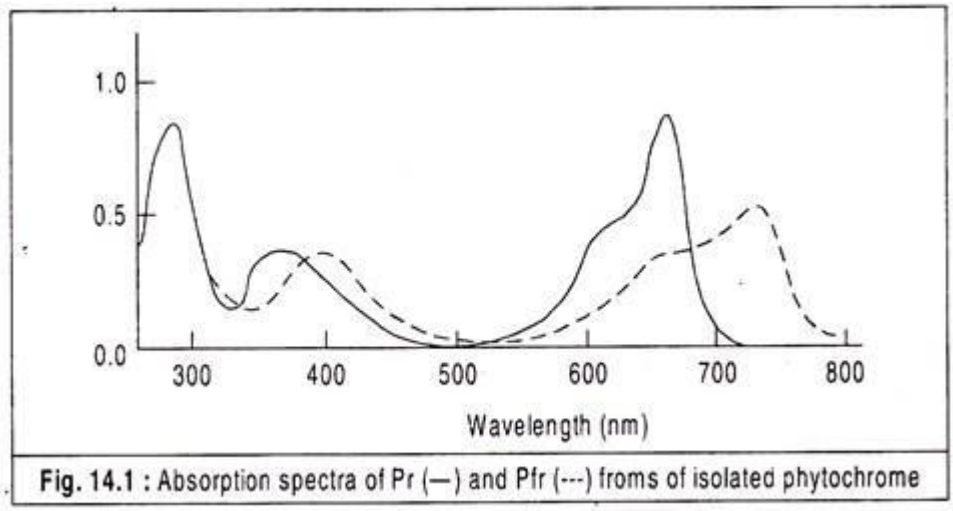
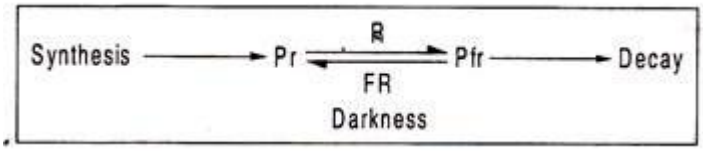


Fig. 14.1 : Absorption spectra of Pr (—) and Pfr (---) froms of isolated phytochrome

Besides photochemical conversions, non-photochemical reactions also occur in vivo. Thus, Pfr may undergo dark reversion to Pr. Since natural white light acts like R, phytochrome will remain mainly in the Pfr form at the end of the day. It has been observed that after several hours of .darkness, plants become sensitive to R indicating that Pfr is present in a large amount.

Thus, it is inferred that Pfr is converted spontaneously to Pr in darkness. Phytochrome decay or destruction is also a dominant irreversible process in a seedling, which is the thermo-chemical transformation of Pfr to an inactive form.

Thus, the model of phytochrome action including synthesis, dark reversion and decay can be presented as:



Structure and Biosynthesis of Phytochrome:

Phytochrome is a soluble chromo-protein with a molecular mass of 250 kDa it occurs as a dimer made up of two subunits, each of 125 kDa. Each subunit consists of two components — light- absorbing pigment molecule, chromophore and a polypeptide chain, Apo protein. The Apo protein monomer has a molecular mass of 125 kDa.

Apo protein and chromophore together make up the holochrome. The chromophore is a linear tetrapyrrole similar to phycocyanin termed phytochromobilin and it is a ring attached to the protein through thioether-linkage to a cysteine residue.

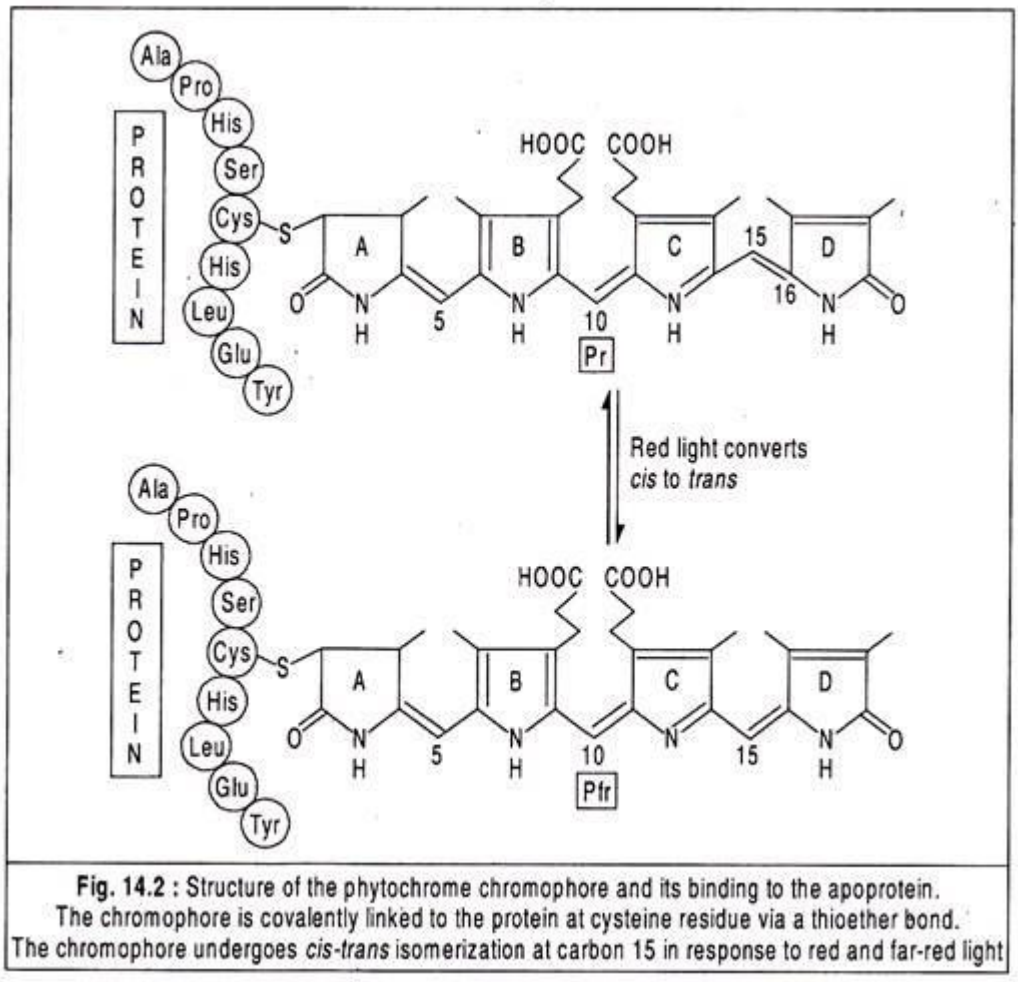


Fig. 14.2 : Structure of the phytochrome chromophore and its binding to the apoprotein. The chromophore is covalently linked to the protein at cysteine residue via a thioether bond. The chromophore undergoes *cis-trans* isomerization at carbon 15 in response to red and far-red light

The principal difference between the Pr chromophore and the Pfr chromophore appears to be a cistrans isomerization of the methane-bridge between rings C and D. The absorption of red light provides the energy required to overcome a high activation energy for rotation around the double bond. There is further evidence that the protein also undergoes photo chemically induced conformational changes.

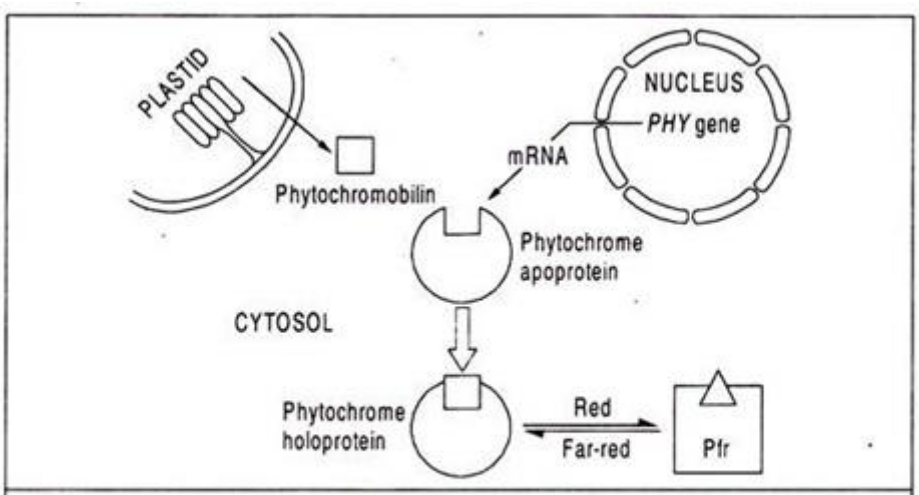


Fig. 14.3 : Phytochromobilin is synthesized in plastid and released into the cytosol, where it assembles with apoprotein to form phytochrome holoprotein. Both chromophore and protein undergo conformational changes (After Kendrick *et. al.*, 1997)

Phytochromobilin is Synthesized in Plastids:

Phytochrome Apo protein alone cannot absorb red or far-red light. Light can be absorbed only when the polypeptide is covalently linked with phytochromobilin to form the holoprotein. Phytochromobilin is synthesized within plastids.

After synthesis, it leaks out of the plastid into the cytosol. Assembly of Apo protein with chromophore is autocatalytic, that is, it occurs spontaneously when purified Apo protein is mixed with purified chromophore in test tube, for which no cofactors are necessary. Assembly in vivo of these two components is also autocatalytic (Fig. 14.3).

(a) Phytochrome is Encoded by a Multi-gene Family:

Complementary DNA (cDNA) copies of mRNAs were isolated from oat and zucchini (Cucurbita pepo) seedlings. Using these clones as probe, five structurally related phytochrome genes were identified in Arabidopsis. This gene family is known as PHY, and its 5 individual members are PHYA, PHYB, PHYC, PHYD and PHYE.

The Apo protein without chromophore is also called PHY, and the holoprotein with chromophore is called phy. Phytochrome sequences from other higher plants are named according to their homology with Arabidopsis PHY genes.

(b) Phytochrome Controlled Responses:

Phytochrome is the photoreceptor involved in many developmental responses of plants to light. It is involved as a light detector and also in the measurement of light duration.

The regulatory effects of light on plant growth and development are visualized most prominently at two stages in the life cycle of the plant — firstly, at the stage of seed germination and seedling development, and secondly, at the stage of transition from the vegetative to the flowering phase.

For the sake of convenience, these diverse photo-morphogenetic responses can be classified into the following types:
Fast responses and slow responses.

Type I: Fast Responses:
The type I responses include those processes in which the quantum energy absorbed by the plant is transduced to another form of energy.

Examples of this type of essentially energy-transducing responses include leaf movement of Mimosa and chloroplast movement in Mougeotia, Other examples are surface potential changes, membrane potential changes and ion fluxes. These phenomena are relatively rapid, occurring on a time scale of seconds and minutes.

Type II: Slow Responses:
The rates and activation of certain aspects of growth and development are switched on or modulated under the influence of the quality of light (red or far-red). Examples of type II responses include stem elongation, seed germination, hook opening, leaf expansion, flower initiation and pigment biosynthesis.

Type II responses are relatively slow responses occurring on a time scale of hours and days. The phytochrome molecule is thought to act as a photo chrome sensor that controls the photo-morphogenetic machinery of plants.

(c) Variation in Lag Time, Escape Time and Light Quanta for Phytochrome Responses:
Morphological responses to the photo-activation of phytochrome may be visually observed after a lag time ranging from a few minutes (chloroplast rotation in green alga Mougeotia) to as long as a few weeks (flower initiation). It has further been established that red-light induced effects are reversible by far-red light only for a limited period of time after which the response escapes from the photo-reversible control.

Not only the lag and escape times differ in diverse phytochrome responses, but different amounts of light (fluence) are required to induce them. The amount of light is termed **"fluence"** which is defined as the number of photons per unit surface area. Each phytochrome response is characterized by a specific range of light fluences over which the magnitude of response is proportional to the fluence.
These responses can be categorized into three major groups based on their sensitivities to fluence, viz., (a) very low fluence response (VLFR), (b) low fluence response (LFR) and (c) high irradiation response.

(i) Very Low Fluence Responses (VLFRs):
Examples can be provided by Arabidopsis seeds, which can germinate with very low red light. The reciprocal relationship between fluence and time, known as the Bunsen-Roscoe law of reciprocity is valid in case of VLFRs, which, however, fail to show reversal control by light.

This means that a response can be induced either by brief pulse of red light that is quite bright or by a very dim light for a longer duration. Another point of interest is that far-red cannot reverse VLFRs. The reason is that about 3% of the total phytochrome remaining after far-red exposure is sufficient to induce VLFRs.

(ii) Low Fluence Responses (LFRs):
They exhibit characteristic induction with red light and reversion with far-red light. The law of reciprocity, i.e., light-induced response is a function of total fluence (fluence rate x irradiation time) and independent of the fluence rate or irradiation time holds for LFRs. Such responses include classic red/far-red photo-reversible responses, such as promotion of lettuce seed germination and leaf movement.

(iii) High Irradiation Responses (HIRs):
Some photo-morphogenetic responses require prolonged or continuous exposure to light of high irradiance and are proportional to irradiance, but the reciprocity law is not followed here.

Examples of HIRs are:
(i) Anthocyanin synthesis in dicot seedlings and apple skin

(ii) Inhibition of seedling elongation (hypocotyl)

(iii) Flower induction

(iv) Plumular hook opening

(v) Cotyledon expansion in mustard

(vi) Ethylene production to Sorghum

(vii) De-etiolation of seedlings. However, the effect is not photo-reversible. The reason that these responses are called high irradiance responses (HIR) rather than high fluence responses (HFR) is that they are proportional to irradiance (i.e., brightness of source) rather than to fluence.

Mode of Action in Phytochrome:

Pfr is regarded as the physiologically active form of phytochrome. Conversion of Pr to Pfr by light will produce a particular response depending on the localization of phytochromeand the state of differentiation of the responding cells.

It is also possible that the photo stationary state ratio Pfr/P_{total} acts as a signal perceived by the plant under certain conditions. For example, the HIR response for the inhibition of hypocotyl growth in lettuce can be explained in term of the ratio $Pfr/P_{total} = 0.03$, i.e., 3% Pfr level is necessary for the HIR response.

First step is the absorption of light by the pigment. Then the absorbed light alters the molecular properties of phytochrome, which induces a sequence of cellular events ultimately leading to a change in growth, development or position of an organ. Generally, two consistent lines of evidences are sought to explain the effects of phytochrome.

One is concerned with the Pfr effect on changes in the properties of cellular membranes. The second theme is that Pfr regulates gene expression.

(i) Phytochrome and Permeability:

A rapid photo response is the phytochrome controlled dark closure or folding of the leaflets of Mimosa or Albizzia. The response involves differential changes in turgor in the cells of the pulvinus at the base of each leaflet. The turgor change is associated with the movement of K+ and other ions into ventral cells and out of dorsal cells.

This has led to the suggestion that the primary action of phytochrome is on membrane permeability. Tanada presented further evidence of membrane changes following Pfr action. It was observed that excised barley and mungbean root tips exposed to red light would stick to a negatively charged glass surface (Tanada Effect).

Such light-induced adhesion was found to be red/far-red reversible. It was suggested that the apical part of the root became electro-positive relative to the basal part in response to Pfr.

Rapid phytochrome controlled changes in electric potential have also been measured in the coleoptiles of oat seedlings.

These electrical changes are consistent with a phytochrome induced efflux of ions. Among the different ions transported, $Ca2^+$ helps in transducing photo-activation of phytochrome into physiological changes.

(ii) Phytochrome and Enzymes:

Seedling photo-morphogenesis is associated with the appearance of enzymes necessary for photosynthesis. NADP-dependent GAP dehydrogenase, a key enzyme associated with leaf chloroplasts, changes in activity in response to red and far-red exposure. An enzyme, which has been extensively studied, is phenylalanine ammonia lyase (PAL).

It is the enzyme that catalyzes the conversion of phenylalanine to coumaric acid and thus initiates the synthesis of compounds like coumarin, lignin and flavonoids including anthocyanin pigments belonging to the class of secondary metabolites (Fig. 14.4).

This enzyme is present in very low concentration in the dark but can be greatly increased on exposure to far-red light. Another enzyme, ascorbic acid oxidase, has been shown to increase by Pfr action.

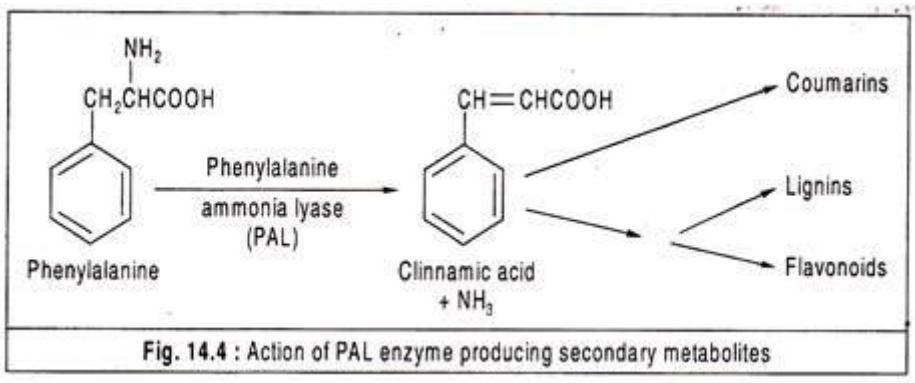


Fig. 14.4 : Action of PAL enzyme producing secondary metabolites

It is not certain whether Pfr stimulates the synthesis of these enzymes or leads to an activation of already existing enzymes.

Some Pfr Responses are mediated by Calcium and Calmodulin:

Calmodulin is a calcium-binding protein and calcium-calmodulin ($Ca2^+$ -CaM) complex may regulate plant responses which include several enzymes like plasma membrane-localized $Ca2^+$ pump ($Ca2^+$ -ATPase), NAD kinase and enzymes (kinases and phosphatases) that cause phosphorylation and activation of other enzymes.

Several lines of evidence indicate that $Ca2^+$ can mediate phytochrome responses. Calcium uptake into the cells is increased by Pfr and some Pfr-stimulated enzymes are also stimulated by calmodulin (CaM).

By using Ca^{2+} -ionophore, a chemical agent that promotes Ca^{2+} uptake into cells, some phytochrome responses can be induced in darkness. It is suggested that chemically induced uptake of Ca^{2+} into cell can mimic Pfr responses and acts as a substitute for red light. It is tempting to conclude that calmodulin is the agent that transduces Ca^{2+} entry into physiological responses.

(iii) Intracellular Localization: Phytochrome Bound to Membrane:

In higher plants, 75% of the total amount of phytochrome is localized in protoplasts, while the remaining 25% divided equally between the vascular and epidermal tissues. Attempts have been made to locate phytochrome within the cell by micro spectrophotometry and immunocytochemical methods.

Purified phytochrome is injected into an animal to produce a highly specific antiserum, which is then used to locate phytochrome in tissue sections. These studies indicate that Pr form of phytochrome is diffusely distributed, whereas the Pfr form is present in a discretely localized pattern. Phytochrome Pr is a stable protein, whereas Pfr can bind and fuse to membrane, thus modulating membrane activity.

In contrast to higher plants, many Type-I responses (quick responses) in simpler organisms are mediated by membrane-bound phytochrome. For example, phytochrome in Mougeotia, a filamentous green alga, is membrane bound. The cells of this alga contain a single ribbon-like chloroplast, which shows movement controlled by phytochrome (Fig. 14.5).

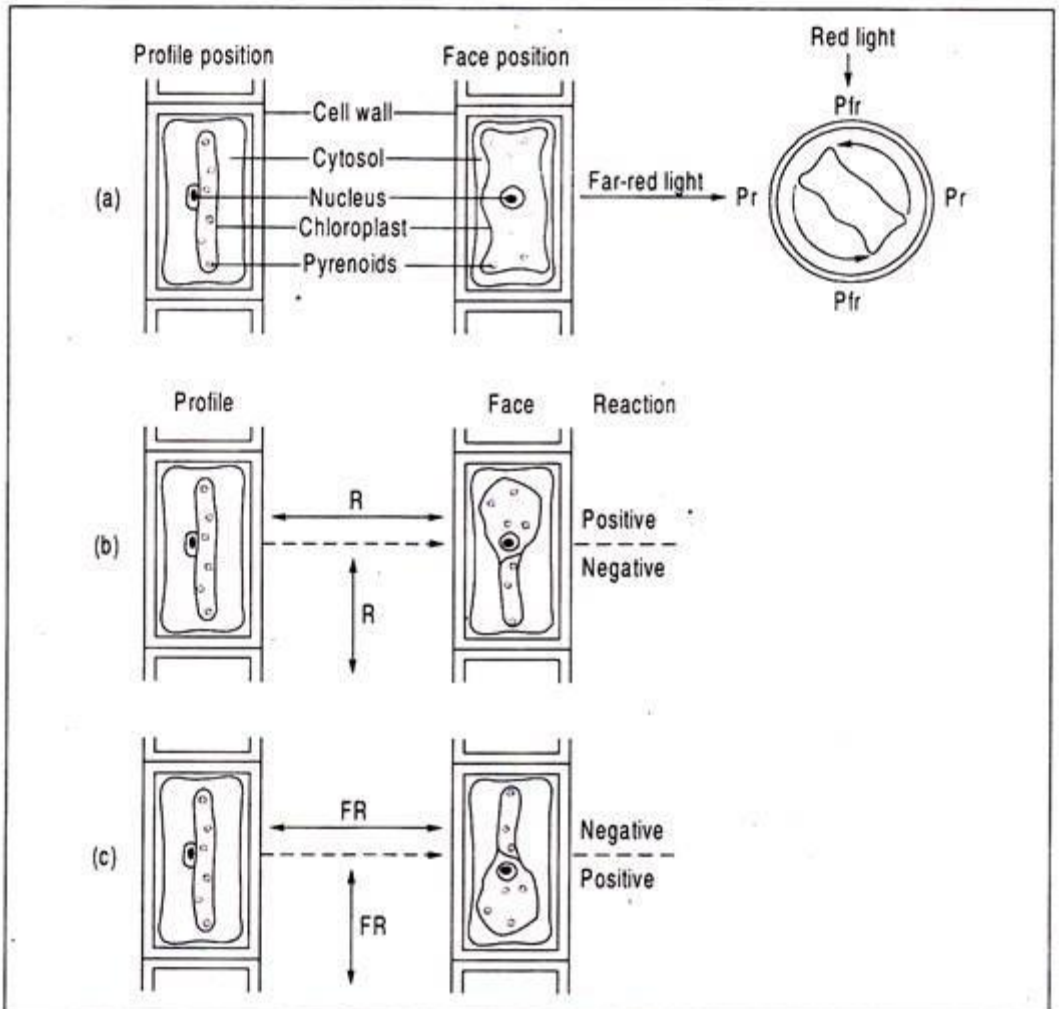


Fig. 14.5 : Phytochrome control of chloroplast orientation in cells of algae *Mougeotia*

(a) *Left and centre* : Cells shown in profile and face positions (surface view). *Right* : Change in orientation of chloroplast after exposure to red and far-red light (cross-sectional view).

(b) *Left* : Cells in dark with chloroplast in profile position. *Right* : Orientation of chloroplast after red light exposure. R incident perpendicular to chloroplast (upper half) causes rotation. There is no rotationinin lower half receiving R in parallel direction.

(c) *Left* : Cells in dark with chloroplast in profile position. *Right* : No rotation when FR is perpendicular to chloroplast (upper half), rotation of lower half after exposure to FR parallel to chloroplast.

When red light falls perpendicular to the long axis of the cell, it is preferentially absorbed at the front and backsides of the cell converting Pr to Pfr form. As Pfr builds up at the front and back of the cell, the chloroplast can rotate about its long axis and can respond to the incident radiation by orienting perpendicular to the direction of light (face position).

On the other hand, red light falling parallel to the long axis of the cell is not effective for chloroplast movement, which persists in profile position. In this case, the red light effect can be reversed by far-red light proving that phytochrome is implicated in the response. Rotation can also be induced by far-red light, but the chloroplast should remain parallel to the source of far-red light.

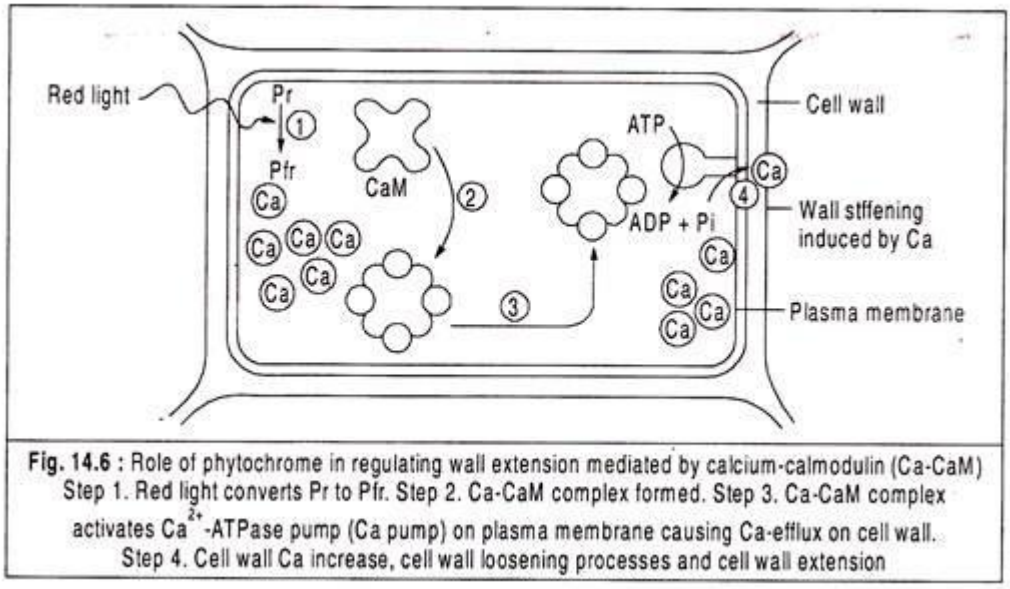


Fig. 14.6 : Role of phytochrome in regulating wall extension mediated by calcium-calmodulin (Ca-CaM) Step 1. Red light converts Pr to Pfr. Step 2. Ca-CaM complex formed. Step 3. Ca-CaM complex activates Ca^{2+}-ATPase pump (Ca pump) on plasma membrane causing Ca-efflux on cell wall. Step 4. Cell wall Ca increase, cell wall loosening processes and cell wall extension

(iv) Inhibition of Internode Extension:

A rapid visible response to photo activation of phytochrome is the inhibition of internode extension of growing seedlings. Ca^{2+} and CaM dependent cellular events can be linked to red light induced inhibition of internode extension. When calcium concentration of cell wall increases, cell-wall loosening processes are inhibited leading to wall stiffness, which in turn inhibits extension.

Most of the cellular calcium is bound to cell structures and organelles like vacuole, mitochondria and endoplasmic reticulum. Initially, Pfr promotes the release of Ca^{2+} from these structures into the cytoplasm and thus causes a transitory increase in cytosolic calcium. Phytochrome regulation of wall extensibility based on CaM is thought to occur in a stepwise manner.

First, phytochrome is activated by red light converting Pr to Pfr. This is followed by Pfr-induced CaM activation. Then Ca^{2+} -CaM complex stimulates plasma membrane-bound Ca^{2+} – ATPase which pumps Ca^{2+} out from cytosol into the walls.

(v) Phytochrome Stimulates Gene Expression:

It is generally accepted that most living cells of a particular plant contain all the genetic information in the form of DNA characteristic of that plant. Differences amongst cells arise from differential gene activity, genes being **'turned off'** and **'turned on'** during development. Role of phytochrome in the major developmental events obviously suggests that changes in gene expression are involved.

Studies on phytochrome regulation of gene expression are so far concerned with nuclear genes encoding for mRNAs of chloroplast proteins. Two such proteins have been investigated. These are — (i) the small subunit of Rubisco, and (ii) the light-harvesting chlorophyll binding protein (LHCP) associated with light-harvesting complex of PS II.

These two proteins play an important role in chloroplast development and greening. Rubisco is the key enzyme in photosynthesis, which catalyses the addition of a CO_2 molecule to an acceptor molecule RuBP. It is an oligomeric protein, consisting of eight large subunits (LSU) and eight small subunits (SSU).
The LSU is encoded by the chloroplast genome and synthesized in the chloroplast. The SSU is encoded in the nuclear genes and synthesized in the cytoplasm.

Similar to SSU, the Apo protein of LHCP, known as chlorophyll a/b binding protein is encoded in the nucleus, synthesized on cytoplasmic ribosomes, and transferred into the chloroplast. The corresponding genes for these proteins are rbcS (rubisco Small) and cab (chlorophyll a/b).

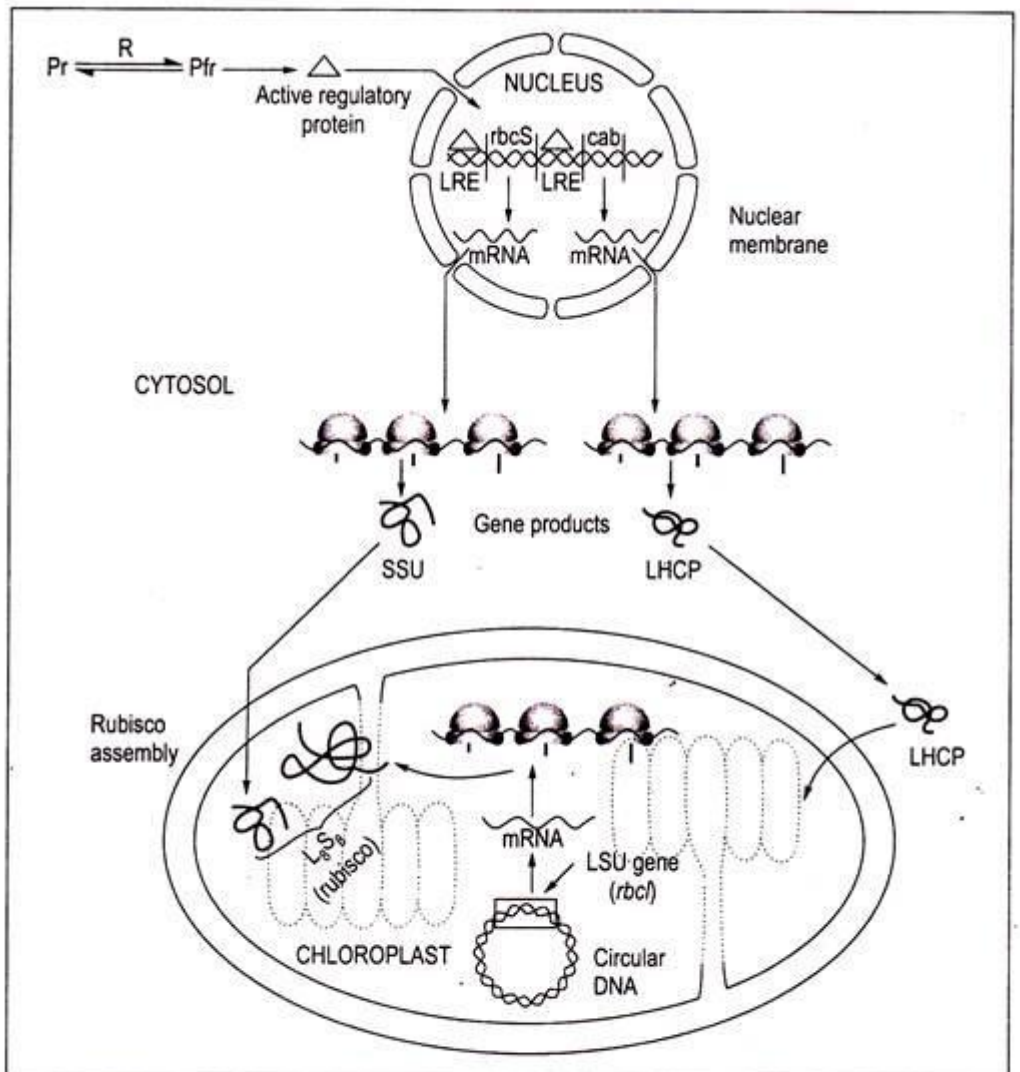


Fig. 14.7 : Phytochrome regulation of rbcS and cab genes. Red light converts Pr to Pfr. Pfr activates regulatory proteins in cytosol. Such proteins migrate to nucleus and bind to specific light-regulated elements (LREs) in promoter regions of rbcS and cab genes. Transcription is stimulated, leading to enhanced synthesis of gene products, SSU of Rubisco and light-harvesting chlorophyll protein (LHCP)

In an early work, Rubisco protein of barley seedlings was found to be stimulated by light. Recently, translation studies of mRNA isolated from duckweed (Lemna gibba) have been made by Tobin and her associates in the United States, who have shown that mRNA levels for SSU protein and chlorophyll a/b binding protein increase after exposure of dark-grown seedlings to light.

Only I min light exposure is needed to increase mRNA and the effect is reversed by far-red light. It has been confirmed that Pfr acts to increase the rate of transcription of these two genes for the small subunit of Rubisco and the chlorophyll a/b binding protein.

Using modern molecular techniques, it has been established that the promoter region of rbcS gene is light-regulated.

Promoters are DNA sequences located upstream on 5′ flanking side of a gene, which function in the regulation of transcription. Such regulatory sequences in the promoter region are called c/s-acting DNA. Recently, promoter regions of rbcS gene from peas have been shown to contain c/s-acting elements involved in light regulation.

It has been proposed that red light converts Pr to Pfr. Then Pfr activates one or more regulatory proteins. It is curious that phytochrome itself has no DNA-binding capacity. So, the activated regulatory proteins behave as trans-acting factors, which are DNA-binding proteins that bind to c/s-acting DNA sequences and regulate gene transcription.

Such DNA sequences known as light regulated elements (LREs) have been identified in the promoter region for two genes — rbcS abd cab.

Thus, the activated regulatory protein then binds to specific light regulated element (LRE) and stimulates transcription of the gene, leading to an enhanced synthesis of gene products, SSU of Rubisco and light harvesting chlorophyll protein (LHCP).

These proteins contain transit peptides that facilitate their entry into chloroplasts. After entering the chloroplast, SSU combines with LSU to form holoenzyme. The other protein LHCP is associated with PS II in thylakoid membrane.

(vi) Phytochrome Inhibits Gene Expression:

Contrary to the genes stimulated by phytochrome, there are at least two genes where Pfr causes a decrease in transcription. One such negatively regulated gene encodes for NADPH-protochlorophyllide oxidoreductase, the enzyme that catalyzes reduction of protochlorophyllide to chlorophyllide. A decrease in the level of translatable mRNA is thought to be the cause of decrease in activity of this enzyme in light.

The other negatively regulated gene is the gene that encodes for phytochrome itself, meaning thereby that phytochrome regulates the expression of its own gene. When Pr is converted to Pfr by red light, phytochrome mRNA decreases by decline in the rate of transcription.

Thus, phytochrome genes are auto-regulated by some form of feedback inhibition. Such phytochrome-induced repression of phytochrome gene expression is a factor, which possibly explains a rapid decrease in total phytochrome when dark-grown plants are transferred to light.

(vii) Crypto Chrome: Blue-Light Responses:

A large number of plant responses to blue light have been known for a long time. Blue-light signals are generally used by a plant in many responses that provide means to sense the presence of light and its direction. The specific blue light responses of higher plants include phototropism, stomatal movement, inhibition of hypocotyl elongation, pigment biosynthesis and gene activation.

Such plant responses to blue light are quite distinct from phytochrome-induced responses.

These responses have a characteristic action spectrum showing a **"three-finger"** structure in 400 to 500 nm that is not observed in the absorption properties of either phytochrome or chlorophyll (Fig. 14.8).

For example, phototropism, which involves bending towards light, cannot be induced by red light. Likewise, inhibition of hypocotyl elongation by blue light is a specific blue light response in 400-500 nm, independent of phytochrome activity (Fig. 14.9).

It is true, however, similar response in 700-750 nm indicates phytochrome-dependent response. Another distinction between red light (i.e., phytochrome) and blue light inducing the same response is based on the relative rapidity. The inhibitory effect of phytochrome on hypocotyl elongation occurs within 15 to 90 min, whereas the blue light response is quite fast requiring only 15 sec.

(viii) Phytochrome and Flowering Response in Short-Day Plants (SDP):

In several SDP like Xanthium, soybean, Amaranthus, Chrysanthemum, Pharbitis and Chenopodium, night break by R inhibits flowering and FR is effective in reversing a red light (R) night break. So, it is assumed that Pfr which is produced by a R night break reacts to inhibit flowering in SDP (Table 13.2).

It has also been observed that irradiation with R or FR establishes widely different photo stationary states of 0.75 and 0.03, respectively, yet both when applied singly prevent flowering.

Thus it is clear that inhibition of flowering is not proportional to the amount of Pfr. but it depends on the maintenance of the Pfr level for sufficient time above some threshold level.

A relatively short exposure with R is effective in preventing flowering in SDP because it induces a high photo stationary state (Pfr/P = 0.75). Thus, when the plants are returned to darkness, it takes a relatively long time for Pfr to decay below the threshold level.

On the contrary, relatively long exposure to FR is required to prevent flowering because the proportion of Pfr to total phytochrome (Pfr/P) is low, and so on return to darkness, decay below the threshold value occurs rapidly, it is also interesting to note that the possibility of re-promotion of flowering by FR is reduced with each successive R/FR cycle (Table 13.2).

In these treatments, the dark intervals between the light flashes are sufficiently long to permit Pfr to fall below the threshold value leading to the maintenance of Pfr above the optimum level required for flowering.

There is evidence that phytochrome controls flowering response in SDP not only in the dark period, but also during the day period. In Xanthium and Pharbitis, the light quality during a light period intervening between a subcritical dark period and an inductive dark period strongly favour flower formation. Action spectra indicate that the maximum promotion is at 660 nm and inhibition at 730 nm.

It is likely that phytochrome is the photoreceptor since there is evidence of R/FR reversibility. Again, in dark-grown a seedling of Pharbitis, a prior exposure to light with either long or brief R irradiation is required if a single inductive dark period is to be effective for flowering. The effect of R irradiation is reversible with FR, hence it indicates that phytochrome is the photoreceptor.

(ix) Phytochrome and Flowering Response in Long-Day Plants (LDP):
It is generally believed that phytochrome controls opposite reactions in SDP and LDP. This means that light interruptions of long nights which inhibit flowering in SDP should promote flowering in LDP. Basically this is true but very brief interruptions of long nights do not always promote flowering in many LDP. Generally, a long night break is effective for LDP.

The light quality during prolonged night breaks shows that either FR or R and sometimes a mixture of R and FR is effective in promoting flowering in LDP. It has further been demonstrated that flowering in various LDP is more enhanced with incandescent lamp with a high proportion of FR energy than with fluorescent lamps having less FR energy.

In crucifers, neither R nor FR shows marked acceleration of flowering. Here blue light is most effective either as night interruption or as day extension as initially shown by early reports of Funke and Wassink et al.

Just as dark interruption with light is effective in promoting flowering, a simple day length extension has also been found to be effective. In this case, R alone or fluorescent light given during the extension period is not as effective as FR or a mixture of R and FR or incandescent light.

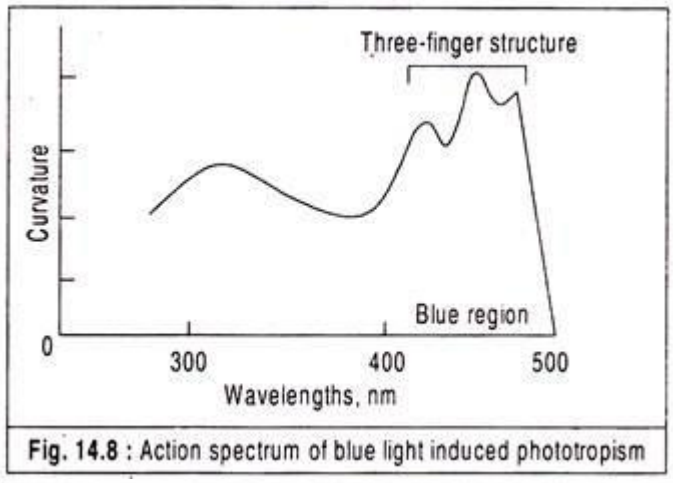


Fig. 14.8 : Action spectrum of blue light induced phototropism

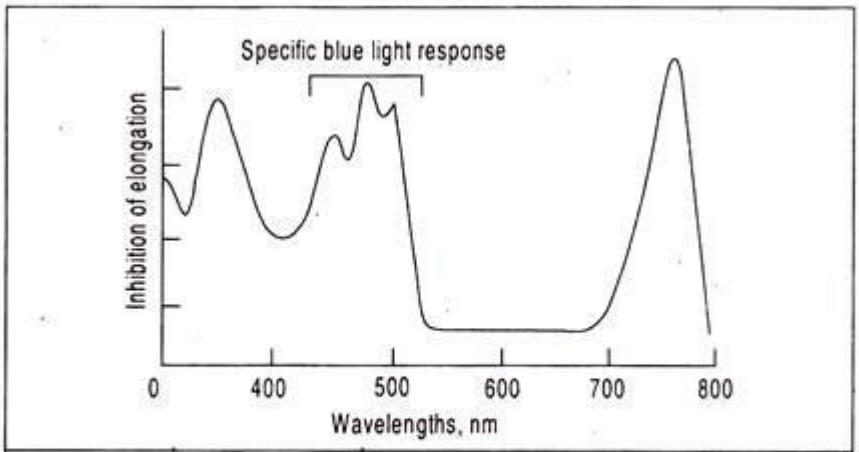


Fig. 14.9 : Action spectrum for inhibition of stem elongation of dark grown seedlings. Three-finger structure in 400-500 nm is blue light response. Response in 700-750 nm indicates phytochrome-mediated response

The exact identity of the blue light photoreceptor is not yet known hence the name crypto chrome has been given which implies a **"hidden pigment"**. In view of the similarity between the absorption spectra of β-carotene and riboflavin and the action spectrum for phototropism. It has been proposed that carotenoids and flavins are the probable photoreceptors or crypto chrome for blue light responses. Carotenoid zeaxanthin has recently been implicated as a blue light photoreceptor.

It has photo-protective role and a role in signal transduction. Absorption spectrum of zeaxanthin closely matches the action spectrum for blue light stimulated stomatal opening. The most common flavins occurring widely in living organisms are riboflavin and its two nucleotide derivatives, flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD).

Blue light has been shown to regulate gene expression. The gene that codes for the enzyme chalcone synthase (for flavonoid biosynthesis) is blue light regulated, while the gene for the small subunit of Rubisco and for the, proteins that bind chlorophylls a and b are not only sensitive to red light (i.e., phytochrome response), but also sensitive to blue light.

Another enzyme, glutamyl semialdehydeaminotransferase, a key enzyme in chlorophyll biosynthetic pathway, is encoded by GSA gene which is sensitive to blue light. Recently, a gene has been isolated from Arabidopsis, which has been shown to encode the blue photoreceptor that causes inhibition of hypocotyl elongation.

The gene and the corresponding protein have been named CRY 1 (for crypto chrome 1). Two chromophores are likely to be associated with CRY 1. One of them is a flavin (FAD) and the other is possibly a pterin.

In view of the above observations, several hypotheses have been put forward to account for the promotive effect of FR and the requirement of prolonged exposure to light in LDP. It has been argued whether phytochrome is the only photoreceptor involved in photo induction in LDP. Thus, the existence of another pigment, possibly a flavoprotein. has also been postulated to explain the effectiveness of blue light.

It is, however, suggested that flowering in LDP is a type of **'high irradiation response'** (HIR) and it is now widely accepted that phytochrome is the photoreceptor in HIR. Even the responses to blue light may reflect different Pfr requirements and thus no pigment other than phytochrome may be involved.

Phototropism & photoperiodism

Key points

- Plants have a variety of developmental, physiological, and growth responses to light—sometimes only to particular wavelengths of light.
- In **phototropism** a plant bends or grows directionally in response to light. Shoots usually move towards the light; roots usually move away from it.
- In **photoperiodism** flowering and other developmental processes are regulated in response to the **photoperiod**, or day length.
- **Short-day plants** flower when day length is *below* a certain threshold, while **long-day plants** flower when day length is *above* a certain threshold.
- In many plants, photoperiodism is controlled by the overlap between the day length cue and the plant's internal **circadian rhythms**.

Introduction

Almost all plants can photosynthesize, and photosynthesis is key to these plants' survival: it lets them make sugar molecules that serve as fuel and building materials. But plants respond to light—sometimes, to specific wavelengths of light—in other ways as well. These non-photosynthesis-related responses allow plants to adjust to their environment and optimize growth.

For instance, some types of seeds will germinate only when they receive a sufficient amount of light—along with other cues. Other plants have ways to detect if they are in the shade of neighboring plants based on the quality of light they receive. They can increase their upward growth to outcompete their neighbors and get a bigger share of sunshine.

Plant responses to light depend, logically enough, on the plant’s ability to *sense* light. Light sensing in plants involves special molecules called **photoreceptors**, which are made up of a protein linked to a light-absorbing pigment called a **chromophore**. When the chromophore absorbs light, it causes a change in the shape of the protein, altering its activity and starting a

signaling pathway. The signaling pathway results in a response to the light cue, such as a change in gene expression, growth, or hormone production.

In this article, we will focus on two examples of plant responses to light and explore how these responses allow plants to match their growth to their environments:

- **Phototropism** is a directional response that allows plants to grow towards, or in some cases away from, a source of light.
- **Photoperiodism** is the regulation of physiology or development in response to day length. Photoperiodism allows some plant species to flower—switch to reproductive mode—only at certain times of the year.

Let's take a look at how these light responses work!

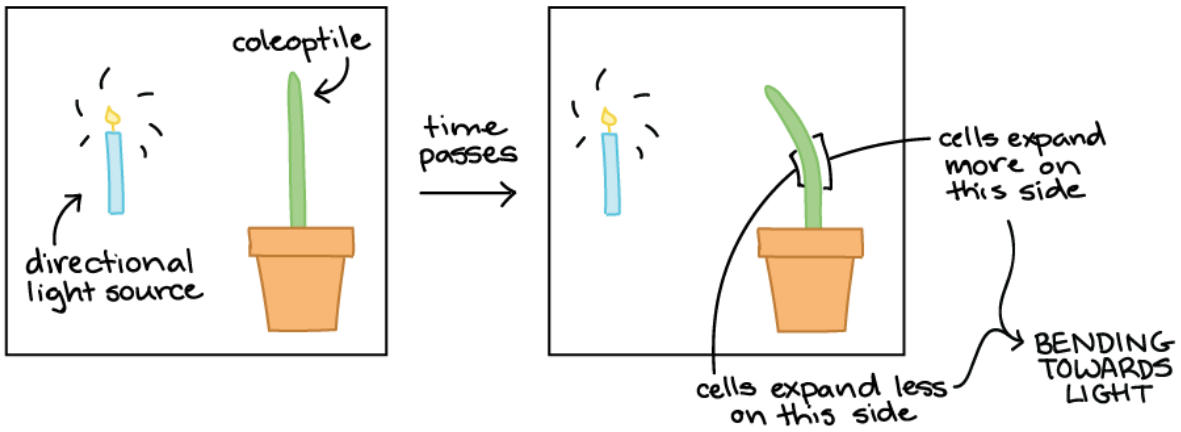
Phototropism

One important light response in plants is **phototropism**, which involves growth toward—or away from—a light source. **Positive phototropism** is growth towards a light source; **negative phototropism** is growth away from light.

Shoots, or above-ground parts of plants, generally display positive phototropism—they bend toward the light. This response helps the green parts of the plant get closer to a source of light energy, which can then be used for photosynthesis. Roots, on the other hand, will tend to grow away from light.^11start superscript, 1, end superscript

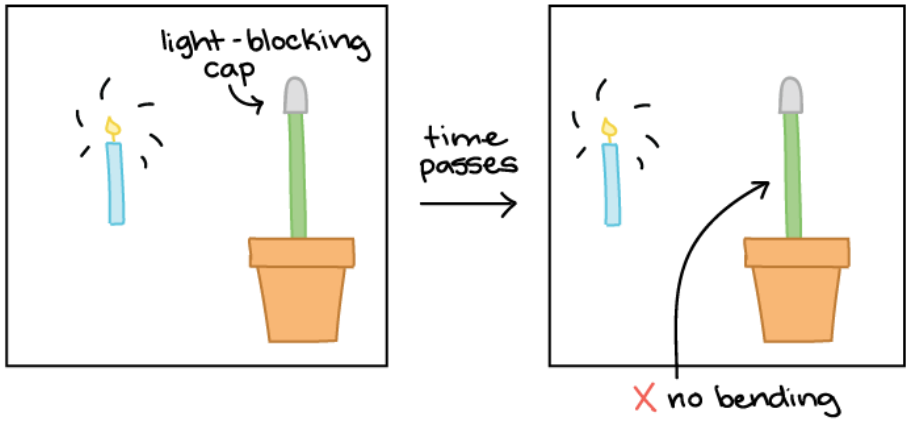
Phototropism involves a mobile signal

In 1880, Charles Darwin and his son Francis published a paper in which they described the bending of grass seedlings towards light. Specifically, they examined this response in very young plants that had just sprouted whose leaves and shoots were still covered by a sheath called the **coleoptile**.



light source (drawn as candle) and a coleoptile in a pot. The pictures shows a straight coleoptile becoming bent toward the light as time passes. The bending is caused by cells closer to the light expanding less than the plant cells facing away from the light.

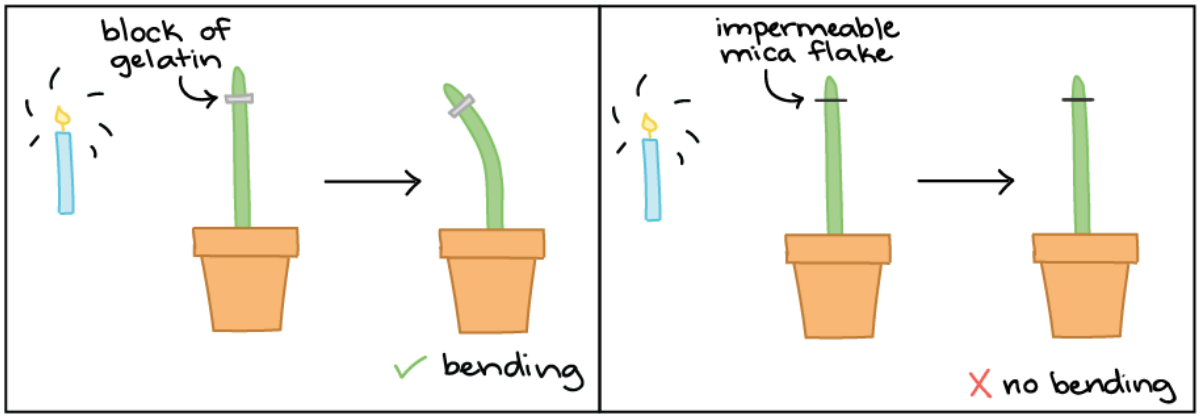
The father-and-son team analyzed the bending response using experiments in which they covered either the tip or the lower part of the coleoptile.^11start superscript, 1, end superscript Through these experiments, they found that light was perceived at the coleoptile's tip. However, the response—bending, at a cellular level, unequal elongation of cells—took place well below the tip. They concluded that some kind of signal must be sent downwards from the coleoptile’s tip towards its base.



light source (drawn as candle) and a coleoptile in a pot with a metal cap covering the very top of the coleoptile. The picture shows a straight coleoptile remaining straight when the metal cap is covering the tip.

In 1913, Danish physiologist Peter Boysen-Jensen followed up on this work by showing that a chemical signal produced at the tip was indeed responsible for the bending response:

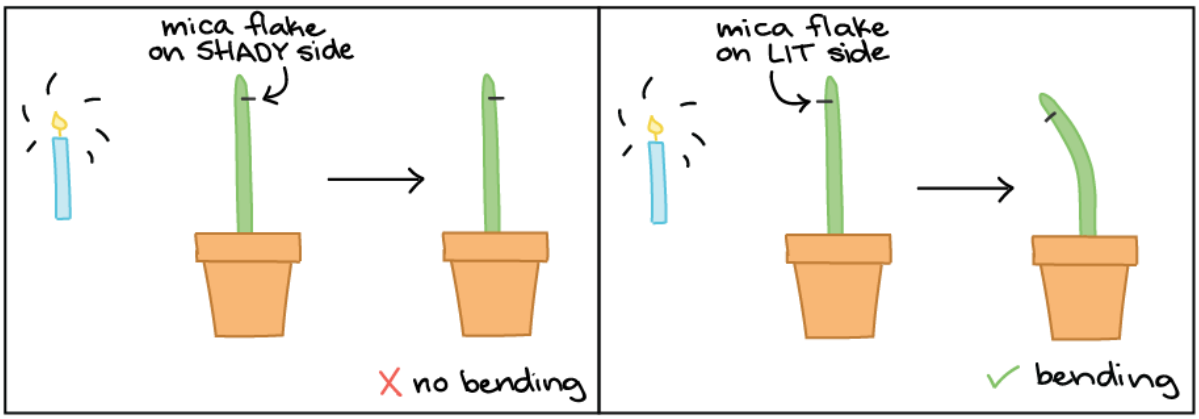
- He first cut off the tip of a coleoptile, covered the cut section with a block of gelatin, and replaced the tip. The coleoptile was able to bend normally when it was exposed to light.
- When he tried the experiment again using an impermeable flake of mica instead of gelatin, the coleoptile lost the ability to bend in response to light.



light source (drawn as candle) and a coleoptile in a pot . The coleoptile on the left has a permeable piece of gelatin separating the tip from the rest of the coleoptile, and it bends toward the light. The coleoptile on the right has a impermeable piece of mica separating the tip from the rest of the coleoptile, and it does not bend toward the light.

Only the gelatin—which allowed a chemical signal to travel through its pores—could allow the necessary communication between tip and base.

Through a variation on this experiment, Boysen-Jensen was also able to show that the mobile signal traveled on the shaded side of the seedling. When the mica plate was stuck in on the illuminated side, the plant could still bend towards the light, but when it was stuck in on the shaded side, the bending response no longer occurred. The results of this experiment also implied that the signal was a growth stimulant rather than a growth repressor since the phototropic response involved faster cell elongation on the shaded side than on the lit side.

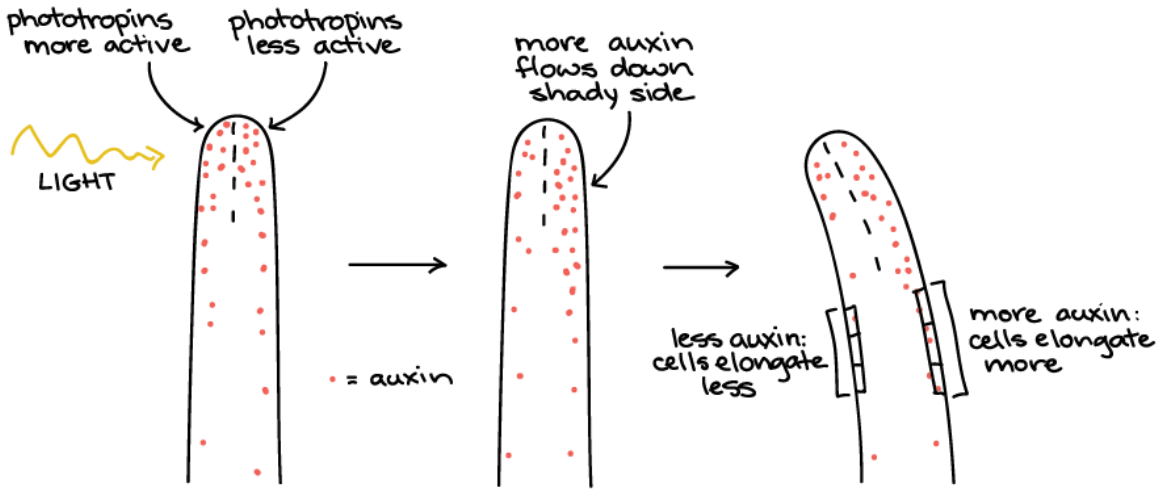


light source (drawn as candle) and a coleoptile in a pot . The coleoptile on the left has a piece of mica separating the part of the tip further from the light from the rest of the coleoptile, and it does not bend toward the light. The coleoptile on the right has a impermeable piece of mica separating the part of the tip closer to the light from the rest of the coleoptile, and it bends toward the light.

Phototropins and auxin

Today, we know that proteins called **phototropins** are the main photoreceptors responsible for light detection during phototropism—the name is a handy reminder of their role! Like other plant photoreceptors, phototropins are made up of a protein bound to a light-absorbing organic molecule, called the chromophore. Phototropins absorb light in the blue range of the spectrum. When they absorb light, they change shape, become active, and can change the activity of other proteins in the cell.

When a coleoptile is exposed to a source of light, phototropin molecules on the illuminated side absorb lots of light, while molecules on the shady side absorb much less. Through mechanisms that are still not well understood, these different levels of phototropin activation cause a plant hormone called auxin to be transported unequally down the two sides of the coleoptile.



Close up of tip of coleoptile showing the plant hormone auxin (pictured as red dots) concentrated toward tip. When light hits one side of the coleoptile, the phototropins are more active on the side with light, causing the auxin to flow down the shady side. The side of the coleoptile with less auxin has less elongated cells, and the side with more auxin has more elongated cells, causing the tip to bend toward the light.

More auxin is transported down the shady side, and less auxin is transported down the illuminated side. Auxin promotes cell elongation, causing the plant to grow more on the shady side and bend in the direction of the light source.

Photoperiodism

Some types of plants require particular day or night lengths in order to flower—that is, to transition to the reproductive phase of their life cycle.

- Plants that flower only when day length drops below a certain threshold are called **short-day plants**. Rice is an example of a short-day plant.^22squared
- Plants that flower only when day length rises above a certain threshold are called **long-day plants**. Spinach and sugar beets are long-day plants.^22squared

By flowering only when day or night lengths reach a certain threshold, these plants are able to coordinate their flowering time with changes in the seasons.

[Is day length the only factor involved?]

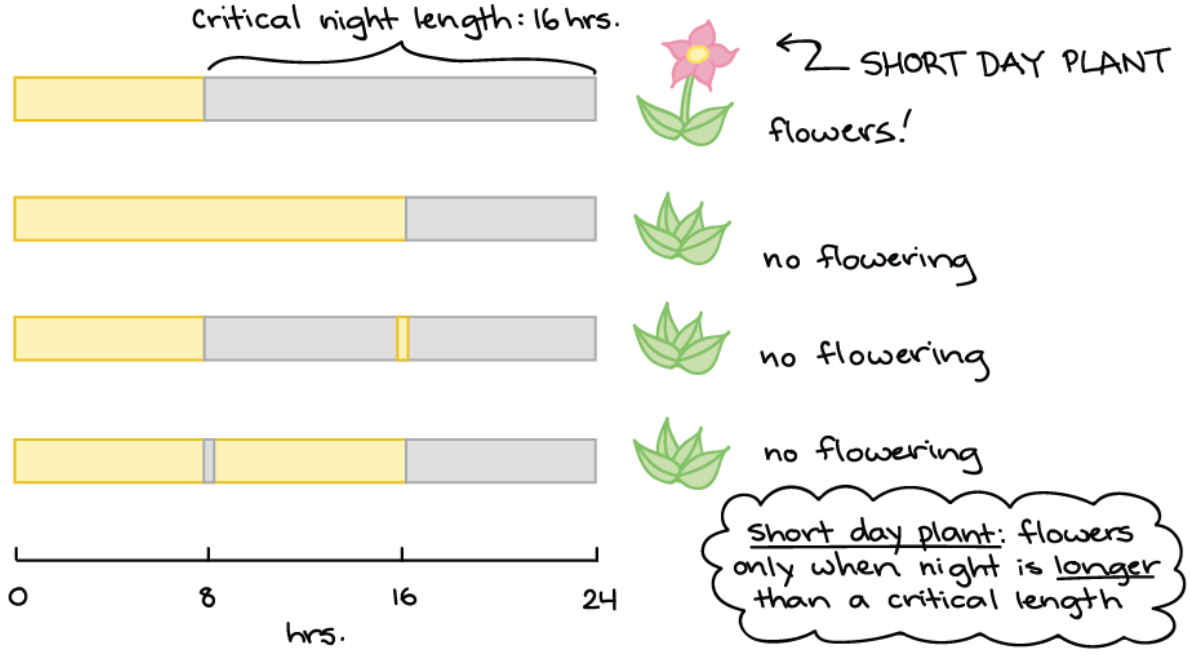
Not all plants are short-day or long-day. Some plants are **day-neutral**, meaning that flowering does not depend on day length. Also, flowering is not the only trait that can be regulated by **photoperiod**—day length—although it's the one that's gotten the most attention from researchers. Tuber formation in potatoes, for instance, is also under photoperiodic control, as is bud dormancy in preparation for winter in trees growing in cold areas.^33cubed

What is the plant actually measuring?

Although we classify plants as short-day or long-day, in some cases, plants may actually be measuring the length of the night. That is, it can be the length of the period of continuous darkness, not the length of the period of continuous light, that determines whether or not the plant flowers.

This is particularly true of short-day plants, whose photoperiodic response is often tightly linked to the length of the night. Typical short-day plants share the following characteristics:^{2,4,5}2,4,5start superscript, 2, comma, 4, comma, 5, end superscript

- They flower when the day is short and the night is long.
- They do not flower when the day is long and the night is short.
- They do not flower when the long night is interrupted by a brief period of light.
- They do not flower when the long day is interrupted by a brief period of dark.



Graph showing the relative hours of daylight vs. night (out of 24 hours) that will cause a short day plant to flower. If the night length is 16 continuous hours, the critical length, the plant will flower.

Image credit: diagram based on similar diagram in Thomas and Vince-Prue^55start superscript, 5, end superscript

What exactly does all that tell us? The pattern in the diagram above means that short-day plants measure the length of the night—the continuous period of darkness—and not the length of the day—the continuous period of light. That is, a short-day plant will only flower if it gets uninterrupted darkness for a length of time that meets or exceeds its flowering threshold. If there is an interruption to the darkness, such as a brief period of light, the plant will not flower, even though the continuous period of light—day—is still short.

The situation changes a bit when we consider long-day plants. Some long-day plants do measure the length of the night, like the short-day plants in the diagram above. However, unlike short-day plants, these long-day plants need the period of darkness to be *shorter than* or equal to a critical length! Long-day plants that measure the night length are said to be **dark-dominant** because it's the period of continuous darkness that's important for flowering.

Many other long-day plant species, however, seem to measure the length of the *day*, not the night, in determining when to flower. These plants are said to be **light-dominant**.^66start superscript, 6, end superscript Scientists think that the majority of long-day plant species are actually light-dominant, while the majority of short-day plant species are dark-dominant.^66start superscript, 6, end superscript

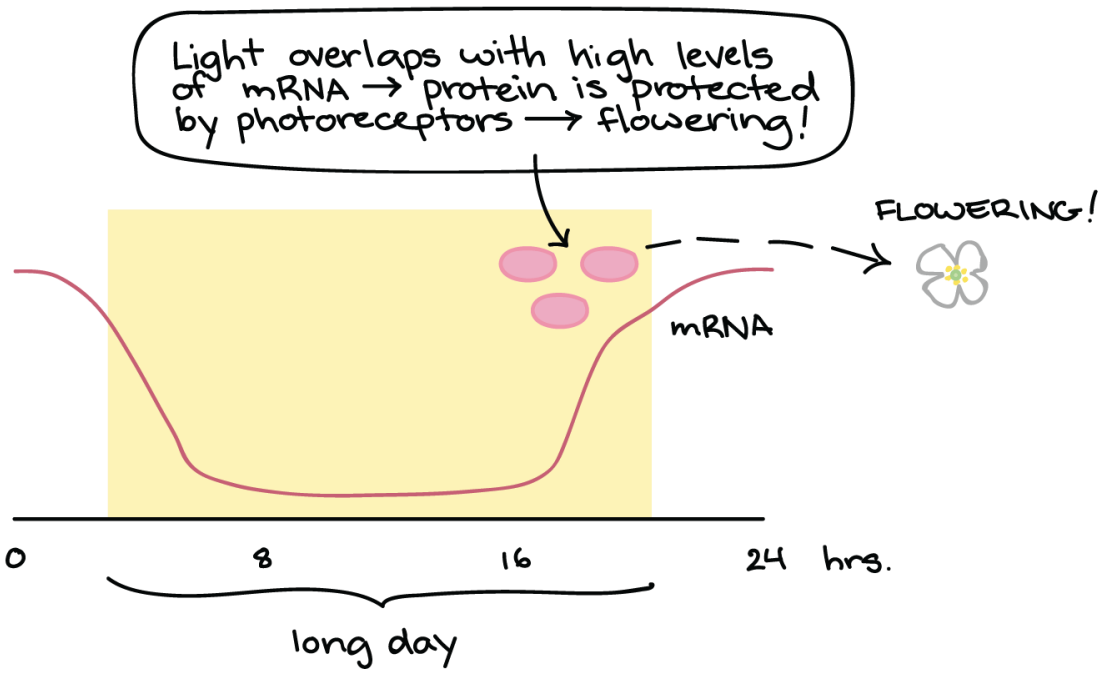
How does the plant determine day or night length?

This is a question plant biologists have been wondering about for decades! Many models have been suggested over the years, but today, most biologists think photoperiodism—at least, in many species—is the result of interactions between a plant's "body clock" and light cues from its environment. Only when the light cues and the body clock line up in the right way will the plant flower.

This model is called the **external coincidence model** of photoperiodism. Its name highlights that an external cue—day length—has to coincide in a certain way with the plant's internal rhythms in order to trigger flowering. These rhythms are **circadian rhythms**, patterns in gene expression or physiology that repeat on a 24-hour cycle and are driven by the plant's internal body clock.

How the external coincidence model works is best understood for the long-day plant *Arabidopsis*, a relative of mustard. In this plant, levels of a specific m\text{RNA}RNAstart text, R, N, A, end text that encodes a flowering induction protein rise and fall on a circadian cycle, with m\text{RNA}RNAstart text, R, N, A, end text levels going up sharply in the evening.^{2,7}2,7start superscript, 2, comma, 7, end superscript [What is mRNA?]

When there is no light in the evening, the high levels of m\text{RNA}RNAstart text, R, N, A, end text don't get the plant very far. That's because the flowering induction protein is usually broken down as soon as it's made. If, however, there's light in the evening—a long day—photoreceptors are activated by the light and jump in to save the protein from degradation. The protein can then build up and trigger flowering.^{2,7,8}2,7,8start superscript, 2, comma, 7, comma, 8, end superscript



Graph showing changing mRNA levels over 24 hours. Areas where the plant is exposed to light are highlighted in yellow. When light overlaps with high levels of mRNA, photoreceptors are activated by the light and protect the flowering induction protein, which can lead to flowering.

Image credit: based on similar diagrams in Lagercrantz^22squared, Figure 4; Kimball^11start superscript, 1, end superscript; and Valverde et al., Figure 4^66start superscript, 6, end superscript

Thanks to this molecular system, the plant flowers only when the days are long—when light extends late enough to overlap with the high m\text{RNA}RNAstart text, R, N, A, end text expression.

[Want to see more molecular details of this model?]

Other models of photoperiodism

Although it seems likely that many plant species use some type of external coincidence model to control flowering and other photoperiod-regulated processes, different plants have different genes and "wiring". It's possible that some plant species have fundamentally different ways of measuring photoperiod and linking this information to developmental changes.

For instance, an older model of photoperiodism, the **phytochrome hourglass model**, does not depend on overlap between circadian rhythms and photoperiod length. Instead, it suggests that phytochromes could act as a clock to measure the length of the night. Although this model is no longer widely accepted, it could potentially be valid for certain types of plants

Vernalization: Definition, Requirement and Importance

1. Definition of Vernalization 2. Site for Vernalization 3. Requirements 4. Mechanism 5. Importance.

Definition of Vernalization:

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

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

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

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

It was found by Lysenko (1928), a Russian worker that the cold requiring annual and biennial plants can be made to flower in one growing season by providing low temperature treatment to young plants or moistened seeds.

He called the effect of this chilling treatment as vernalization. Vernalization is, therefore, a process of shortening of the juvenile or vegetative phase and hastening flowering by a previous cold treatment (Fig. 15.33).

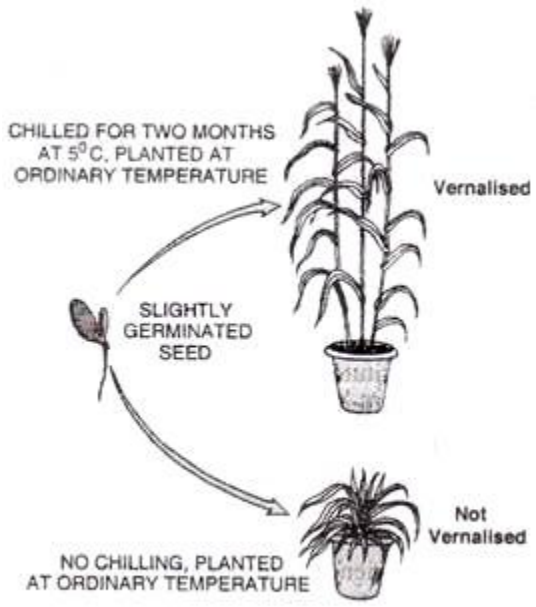


Fig. 15.33. Experiment to show effect of vernalization on Winter Rye.

Site for Vernalization:

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

Requirements of Vernalization:

(i) Low Temperature:

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

(ii) Period of Low Temperature Treatment:

It varies from a few hours to a few days.

(iii) Actively Dividing Cells:

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

(iv) Water:

Proper protoplasmic hydration is must for perceiving the stimulus of vernalization.

(v) Aerobic Respiration and

(vi) Proper Nourishment.

Mechanism of Vernalization:

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

Vernalization prepares the plant to flower. The induction of flowering depends upon the presence of other favourable conditions. Photoperiodism, however, not only prepares the plant to flower but also brings about flowering. Thus, Henbane is a long-day plant which also requires cold treatment. Unless and until both are provided the plant will not come to flower (Fig. 15.34).

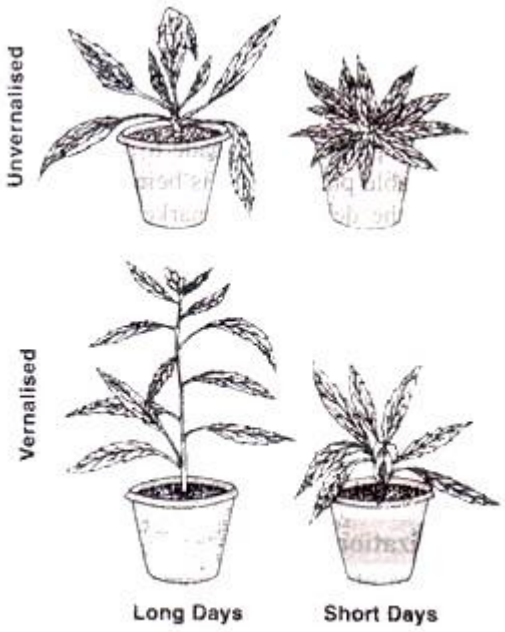


Fig. 15.34. Effect of vernalization and photoperiods on Henbane.

Importance of Vernalization:

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

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

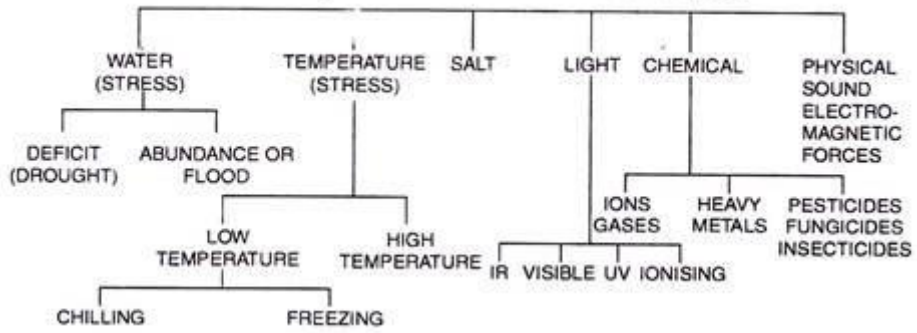
(iii) Kernel wrinkles of Triticale can be removed by vernalization.

How Plants are Damaged through Stress ?

Stress is a mechanical concept and defined as a force per unit area applied to an object. The object develops a strain in response to a specific stress. Compared with mechanical systems, in biological systems it is difficult to define stress precisely. Even though applicable in mechanical terms, its biological connotations is different.

Plants usually yield to any stress condition (Table 28-1) and its reaction may be elastic or plastic. In the former the reaction is temporary and the plant reverts back to its original state. However, in the latter state the plants are deformed and the changes are not reversible. The stress may be immediately made out in the plant or plants may become resistant when exposed to stress conditions.

Table 28-1. Physiochemical stresses that act on plants



This state is called hardening. Sometimes the effect produced is carried over in the subsequent generations. Thus pea or bean plants subjected to low temperature tend to become dwarf and this effect is passed on for several generations.

However, recent years are witnessing studies on genetic basis of resistant strains. Breeders are also making efforts to evolve the genetic lines which are gradually adapted to diverse climatic conditions. The reaction of plants to the stress conditions is highly complex and is manifested in the form of several physiological responses.

The most common stresses to which the plants are exposed to are drought, heat, cold and frost. In addition several other stresses also exist e.g., shade, salt excesses, altitude. In recent times occurrence of excessive pollutants, effluents also give toxic environments to the plants. We shall discuss briefly only some of these stress conditions.

In general, two types of stress resistance are recognized and these are avoidance and tolerance. In the former an internal environment is created within the plant so that its cells are not put under stress. For instance, in a leaf the process of transpiration is built in whereby the leaf is kept cool even though the environmental temperature is high.

Likewise succulents conserve lot of water to avoid internal drought suffrage. In the latter the plant has the capacity to withstand stress. The examples are found in mosses which can endure desiccation conditions. In some species both the characteristics may be present. Plant physiologists are mainly interested in developmental physiological processes which help the plants to tolerate stress conditions.
It is difficult to determine the magnitude of effect of stress in a plant species. Thus in nature duration and intensity of stress are linked together in complex ways and any information from the laboratory conditions may not provide total and complete amount of hardiness. Thus only drought estimates are obtained on hardiness by creating simulating conditions in the laboratory.

Plants experience usually drought which is one of the commonest stresses. Plants have developed several mechanisms to tide over this stress. Development of thick cuticle, sunken stomata, formation of seeds with low water contents, completion of life cycle in short durations by the desert plants are some of the measures adopted by plants against drought.

In some plants water is retained in enough quantities, or the leaves are reduced to scales. It must be said that drought tolerant mechanisms are not well understood. Dehydration leads to loss of water molecules and thus proteins are disrupted.

Water molecule has several functions to perform and one of these is to help keep complex fluids in a stable configuration. Water loss causes concentration of solutes leading to high concentration of cell sap and intercellular fluids cause a greater decrease in the water potential of the fluids.

This causes stress on the protoplasm. Most of the biochemical processes are adversely affected because of the water imbalance. Changes in the cell pH may also be there.

Plants have developed several mechanisms to tolerate drought conditions. One of these is the presence of hydrophilic substances in the protoplasm like high molecular weight proteins, some carbohydrates (e.g., alginic acid). Low molecular compounds like polyhydric alcohols act as hydrophilic compounds.

These attributes are very common in sea weeds which are subjected to high and low tides. Sugars are usually increased in drought conditions in such plants since their presence insolution directly lowers the water potential of cell sap.

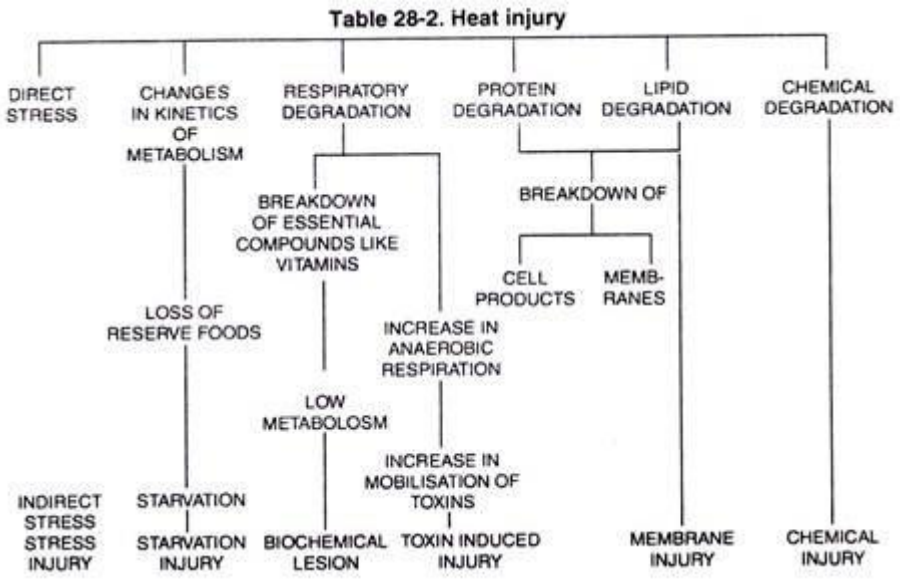
This helps the plant to retain water. This devise helps the plant to conserve water and save the protoplasm from desiccation. In sugarcane high amounts of sugars are present but they are

drought prone while pineapple has less amount of sugars and the drought resistant. Thus the capacity of a plant to bind water to the proteins is very important.

There is also a suggestion that during stress conditions certain resistance proteins appear in a cell and these resist denaturation. Some physiologists are of the view that drought resistance is associated with the protoplasmic elasticity. In general, drought tolerant plants have small cells, high nucleic acid contents, less starch and very high amount of sugars.

Plants responses to heat and their tolerance levels vary (Table 28-2). Leaves may help the plant to avoid heat due to transpiration but the process is negligible.

Table 28-2. Heat injury



Majority of the plant species survive high temperature because of their internal build up. Some of the thermal algae, cactii and several other desert plants experience as high as 70°C and yet survive. High temperature tends to denature proteins and also causes heavy water loses.

One of the attributes is increased enzyme production to compensate for the destruction. It is also reported that in some organisms a rise in temperature slows down some processes and when some compounds are added the process is restarted.

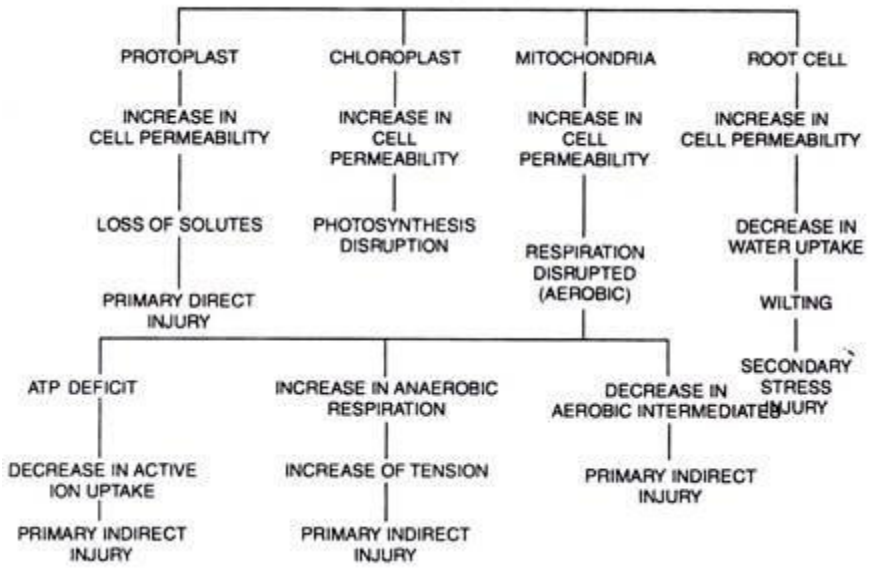
Ascorbic acid, other vitamins are some of these compounds. Recently it has been demonstrated that addition of adenine bestowed tolerance in some plants. In general in plants which are temperature tolerant, process enzymes are more stable to high temperature. In fact in these enzymes some isozymes develop at high temperatures.

In summary it may be stated that most of the plants are heat tolerant because they possess the capacity to produce heat-stable proteins. They also have the ability to replace thermal denatured proteins immediately. However, the precise mechanism of stabilization and synthesis of such proteins is not clear.

Like the heat tolerance, plant species also possess the capability to resist freezing. Several processes may be involved in causing freeze injury (Table-3) to the plant. In general plants growing under tropical climate are more chilling-prone. Hence chilling causes damage to their tissues or organs.

Such plants also are sensitive to low temperature like 12-13°C while low temperature like 0-5-C is lethal. Obviously proteins are sensitive to low temperature. On the contrary, most of the alpine and arctic plants do not experience any damaging effect at this low temperature. In these plants the danger is that their tissues may not undergo water formation in their cells.

Table 28-3. Chilling Temperature



Seeds, pollen, embryos can be stored at low temperature; 190°C obtained through liquid nitrogen. Freezing damage is done in two ways. First, there is formation of ice crystals and the damage is due to mechanical effects. There is disruption of membranes and even cell organization is disturbed.

Second, ice formation reduces the water amount in the cell leading to drought situation. However, the intercellular water has high potential whereas water in the cell cytoplasm or vacuole has nearly negative water Ψ. To begin with it is in the intercellular spaces that the ice crystals are formed and then with the freezing continuing, water leaves the cell cytoplasm. In plants which are freezing-hardy, water tends to remain in the intercellular spaces.

In brief, following is a set of events which take place: Ice crystals formed in the intercellular spaces; protoplast solutes become concentrated due to removal of water from there. There is precipitation of solutes in the protoplast which causes abrupt shifts in the cell pH.

If the temperature is lowered down still further (e.g.—35 to—40°C), all water in the tissues is crystallized. Gradually the crystals enlarge in size and hence there is mechanical damage to the cell. The freezing may be slow or rapid and different plants exhibit different responses.

Chilling-sensitive plants usually have higher proportion of saturated fatty acids (Table 28-4) and hence higher transition temperature. Chilling-resistant species lower proportion of saturated fatty acids and hence lower transition temperatures. During the time the plant gets adjusted to low temperature, the proportion of unsaturated fatty acids enhances and transition temperature decreases.

On the contrary high temperature exposure is accompanied by low soil moisture, high potential transpiration rates etc. High temperature affects membrane and metabolism considerably. For instance several of the enzymes are denatured.

Table 28-4. Ratio of unsaturated : saturated fatty acids of membrane lipids of mitochondria isolated from chilling sensitive and chilling resistant tissues.

***Chilling-sensitive tissues**		
Phaseolus vulgaris	shoot	2.8
Zea mays	shoot	2.1
Lycopersicum esculentum	green fruits	2.8
Chilling-resistant tissues		
Brassica oleracea	buds	3.2
Brassica campestris	root	3.9
Pisum sativum	shoot	3.8

* The data from Lyons *et al., (1964).*

The membrane comes to possess higher proportion of saturated fatty acids in the membrane lipids in the high temperature tolerant species. The fluidity of the membrane increases at high temperature and thus affect permeability and catalytic functions of membrane proteins.

Thylakoid membranes are most sensitive to high temperature damage and thus efficiency of photosynthesis is curtailed. Photosystem II and associated oxygen-evolving complex is grossly affected by high temperatures though activities of Rubisco and other carbon fixing enzymes may get less affected at high temperatures.

Exposure of plants to high temperatures suppresses the synthesis of most proteins but stimulates the synthesis of specific new proteins which are of low molecular mass and known as heat shock proteins (HSPs). Initially discovered in Drosophilla now reported from diverse plants and animals exposed to high temperatures.

There are three classes of HSPs (see Table 28-5) based on their molecular mass. The genetic aspect of these proteins is being well understood but their precise mode of function is still conjuctural. Chaperonin are a class of proteins which direct the assembly of multimeric protein aggregates.

It may be stated that induction of new proteins is not just confined to heat shock, several other stresses like low temperature, water deficit, salinity, anoxia, osomotic stress also induce synthesis of new family of proteins.

Some of these stress proteins resemble HSPs. In summary, it may be mentioned that the synthesis of new proteins appears to be a common response to stress though no universal proteins have been identified.

High concentrations of salt in the plant's surroundings may vary considerably and plant may experience salt stress. In fact large ha of soils are saline. Desert soils have high concentrations of Na, CI, Ca, SO_4 and carbonates.

High salinity is also found near coastal areas, sea shores and even agricultural lands that are heavily irrigated. The salinization of agriculture land has lot of implications on productivity. Salt stress can damage plants at three different levels; alter the soil structure; cause water deficit (physiological drought), and toxic effects of specific ions especially Na, CI.

Molecular biology of salt tolerance is attracting attention recently. Salt-induced gene expression was studied till recently in cell suspension cultures and intact root systems. However, salt tolerant genes have been isolated from rice cv growing in coastal areas and also from mangroves and cloned in rice and tomato.

An increase in the level of osmotin is a striking observation. Cloning of salt tolerant genes will be an important step in raising crop species which grow in saline soils. One of the ways to bolster plant tolerance to salinity is by transferring genes encoding protective proteins or enzymes from other organisms.

Main approaches currently being followed are engineered alterations in the amounts of osmolytes and osmoprotectants, saturation levels of membrane fatty acids and rate of scavenging of reactive oxygen intermediates.

A variety of genes coding for proteins/enzymes involved in stress relief have been introduced into various plant species with reasonable degrees of tolerance to abiotic stresses. These include: low molecular mass osmoprotectants and osmolytes (quaternary amines—betaines), amino acids (proline), sugar alcohols (mannitol, sorbitol) (Fig. 28-5).

Table 28-5 Main heat shock proteins (HSP) found in plants and their possible functions. Number indicate typical molecular mass of proteins.

HSP Family	Possible function
HSP 110	Unclear
HSP 90	Protecting receptor proteins.
HSP 70	ATP-dependent protein assembly, found in cytoplasm, mitochondria, chloroplast
HSP 60	Molecular chaeperons, found in cytoplasm, mitochondria, chloroplasts
LMW HSPs (17-28 KDa)	Functions unknown; form heat shock granules, found in cytoplasm, chloroplasts
Ubiquitin	8 kDa targets other proteins for proteolytic degradation.

There are as many as six theories available to account for the effects of frost and also frost resistance. These are discussed below:

Denaturation of proteins at low temperature:

It is believed that at low temperature in the frost- hardy plants there is formation of low temperature resistant proteins. This may be due to increased concentrations of electrolytes which protect tissue water against its removal by the intercellular ice. Frost-hardy plants have high sugar amount and obviously frost-hardiness involves synthesis of more sugars.

Plant tissues may be made frost-hardy by placing in sugar solution. Thus even though new frost-resistant proteins might be synthesized these proteins must be resistant to high sugar concentrations. Some physiologists believe that shrinkage causes the protein molecules to come together. It is assumed that frost resistance proteins have more hydrophilic bonds.

Thus several factors may be contributory to freezing resistance and these are: formation of low-temperature resistant proteins; dehydration effects; concentration of elctrolytes; high levels of sugars; ice crystal formation and lastly steric effects.

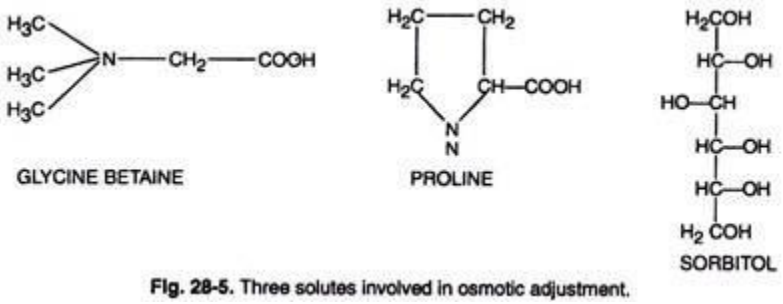


Fig. 28-5. Three solutes involved in osmotic adjustment.

Process of frost hardening is highly complex and much remains to be understood about it. In several plant species it seems to be associated with photoperiod and in some it is essential that tissues experience some pretreatments to attain maximum hardiness. These may include dormancy, photoperiod, etc. Frost hardiness also seems to be associated with the occurrence of metabolism specific inhibitors or even some amount of starvation.

Inadequate light excessive shading of plants causes deformity and starvation. Plants growing in the shade adjust their leaf area, blade thickness, chlorophyll contents, number and orientation of chloroplasts in them. Similarly when $^{14}CO_2$ was provided in high amounts there was strong reaction on the photosynthesis. Several instances are known where radiations have been shown to affect diverse metabolic processes.
Sometimes toxic materials may be present in the soil in large amounts and they affect several mechanisms of the plants. Mangroves are usually referred to as salt regulators since they do not absorb salts but possess the general ability to exclude them from their roots.

On the other hand, there are plant species e.g. Atriplex which are salt accumulators and have cell sap with low and are thus able to absorb salt water of high concentration. Internally such plant species are able to tolerate high concentrations of salt. Such plants also possess special glands on their leaves which excrete high salt contents.

The plants growing at high altitudes also undergo complex stress including climatic conditions. Here much of the plant response depends upon weather conditions, surface microclimate, especially topographical features. In addition radiations also affect their growth.

Plants are also exposed to insects and potentially pathogenic microorganisms though some plants exhibit resistance against disease. Plants adopt several strategies to overcome such adversities. In some plants once the pathogen attacks it, it responds by altering the physical properties of the cell wall and also biosynthesis of secondary metabolites that limit the spread of the attacking pathogen. These responses are known as hypersensitive reactions.

Such reactions are activated by fungi, viruses, nematodes and generally occur outside the pathogen's specificity range. Such hypersensitive reactions are highly complex and are associated with the type of pathogen which attacks it.

Initially there is activation of defense- related genes and synthesis of their products, pathogenesis-related proteins (PR). These proteins include proteinase inhibitors that inhibit proteolytic enzymes secreted by the pathogen and activate chitinase which degrade microbial cell walls. Some genes are activated which synthesize isoflavonoids and other phytoalexins and hence restrict the growth of pathogens.

Lignin, suberin and callose are deposited in the cell wall along with glycoproteins and thus structural support is provided to the cell wall. The invading insects find it tough to perculate such walls. The invaded cells start programmed death resulting in the formation of necrotic lesions at the site of infection. In this way pathogen is isolated, and spread of pathogen is restricted and slowed down.

In the recognition of the potential pathogen it is presumed that a signal detection and transduction chain is involved. The general view is that disease has an underlying genetic basis. Both pathogens and host carry genes that determine the nature of their interaction i.e. whether the disease will occur (virulent) or not (avirulent).

One of the hypothesis is based on gene-for-gene assumption which implies that pathogenic microorganisms carry avirulence genes (avr) while the host plant carries corresponding resistance (R) genes. Disease will take place if the pathogen lacks avr genes or the host plant carries recessive, alleles at the R locus. Thus, hypersensitive reaction is initiated when a matching pair of pathogen avr genes and dominant plant R genes interact.

Several avr genes have been isolated from bacteria and fungi, the specific function of their products is still elusive. There is a possibility that avr gene(s) encode enzyme for the production of elicitors and R gene encodes receptors that recognize elicitors. Elicitors are metabolites which have been isolated from pathogens that evoke a response in host plant.

Several elicitors have been identified and they are usually extracellular microbial products generally associated with cell walls of bacteria or fungi. These are β-glucans, chitosan, arachidonic acid, glycoproteins, polysaccharides, small peptides, pectic fragments.

Recognition of elicitors by the plant cell must take place at the plasma membrane. Several signalling agents have been suggested and these include changes in pH, ion fluxes, transient uptake of Ca^{2+}. Intracellular Ca^{2+} levels appear to regulate expression of defence of response genes.

Thus by activating Ca^{2+} ionophores or blocking Ca^{2+} channels it is possible to activate defence responses. The role of protein phosphorylation and production of active oxygen are also shown to be concerned with elicitor-treated cells. Much remains to be understood regarding the exact role of these signals and their interaction with signal cascade.
These are some secondary metabolites which appear to be associated with the hypersensitive reaction and constitute early warning system, eminating signals to other cell and/or tissues in order to prepare themselves for the secondary infection. The studies have shown that initially the plant reacts to the initial infection by slowly developing a general immune capacity.

This is known as systemic acquired resistance (SAR). This aspect is still in its infancy but salicylic acid appears to be involved in such a signalling pathway (Fig. 28-6). This phenolic acid is secondary metabolite having analgesic properties.

During 1990's a relationship between salicylic acid (SA) and resistance to pathogenes was shown and it was demonstrated that application of SA or its derivative aspirin could induce PR genes expression and enhance. The resistance to TMV. Over the years it has been repeatedly demonstrated that following infection, the level of SA increases in the host and the increase could be as high as 20-fold over the controls.

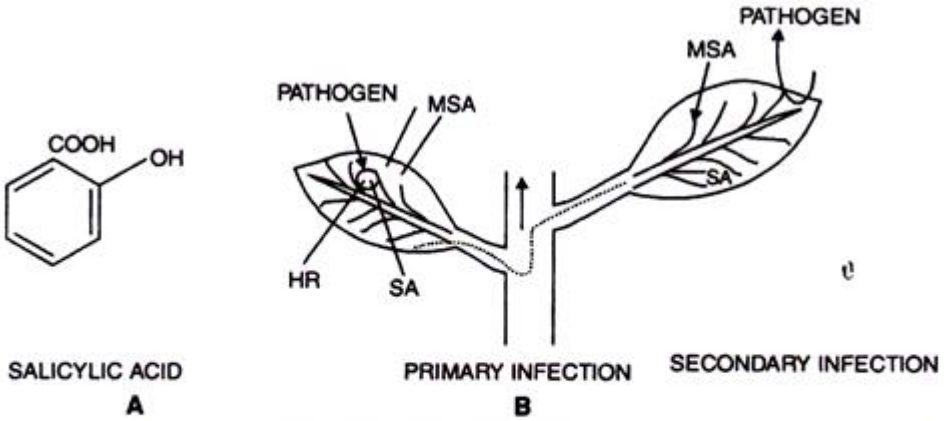


Fig. 28-6 A. The chemical structure of salicylic acid which is implicated in the immune strategies of plants. B. The suggested role of salicylic acid in systemic acquired resistance (SAR). The primary infection by a pathogen stimulates a localized hypersensitive reaction (HR) leading to the synthesis of salicylic acid. This chemical is translocated via phloem to other regions of the plant where it prevents secondary infection by other pathogens. It has also been suggested that salicylic acid may be converted to methylsalicylic acid (MSA) which is slightly volatile and may act as airborne signal.

Such a burst in SA was also followed by the formation of PR proteins. In Arabidopsis plants and also mutants, a parallel between SA, pathogenicity and resistance was demonstrated. On the contrary plants with low levels of SA or such mutants having low levels of SA failed to have SAR. In plants with suppressed phenyllanine ammonia lyase activity the level of SA was low and hence they displayed decreased resistance to the pathogens.

However when the SA was applied externally the resistance was introduced. Evidently, SA appears to have a pivotal role in plant defence responses. Very recent studies point out the

role of jasmonates and their derivatives in imparting insect and disease resistance in plants. Jasmonates are shown to be universally distributed in plants and in actively growing cells/tissues their level is very high.

Jasmonic acid is also reported to induce/activate several genes encoding proteins with antifungal properties. In fact several similarities in the action of SA and jasmonates with respect to insect and fimgal resistance have been suggested though some distinctions have also been demonstrated. It appears that there are two defense mechanisms, one mediated by SA and the other mediated by jasmonic acid.

The precise role of jasmonates in activation of genes is still obscure. However, jasmonic acid is shown to be synthesized from linolenic acid and some authors have suggested its role as a second messenger. Since plant membranes are rich in linolenic acid as phospholipids they may function as elicitors and then bind with a receptor in the plasma membrane.

Thus elicitor-receptor complex activates a membrane-bound phospholipase releasing linolenic acid. Subsequently linolenic acid is oxidized to jasmonic acid which in turn acts to modulate gene expression. It may be stated that action of jasmonates is not restricted to insects or fungi, they modulate several physiological processes including seed germination, pollen germination, vegetative protein storage, root development, tendril coiling, etc. In such processes jasmonates are shown to work in collaboration with ethylene. Some workers have proposed a status of plant hormone to jasmonates.

In recent years environmental pollutants have added another new set of stresses for the plants. Pollution stress is mainly chemical in nature and includes toxic effects of heavy metals, airborne oxides of carbon, nitrogen, sulphur and photochemical products. Many plant species have the potential to detoxify heavy metals by binding them with small sulphur-rich poly-peptides known as phytochelatins.

Plants and Biotic Stresses

the two methods employed by plants to cope with biotic stresses. The methods are: 1. Hypersensitive Response and 2. Secondary Acquired Resistance.

Method # 1. Hypersensitive Response (HR):

On being attacked by insects or a pathogenic microorganism, typically a plant responds with:

(i) Formation of pathogenesis-related (PR) proteins,

(ii) Biosynthesis of phytoalexins,

(iii) Changes in composition and physical properties of cell walls and finally

(iv) Process of programmed cell death wherein the cells immediately surrounding the infection site die rapidly to deprive the pathogen of nutrient supply and thus checking its spread in host plant and leaving a necrotic lesion at the site of invasion. All these responses are collectively known as hypersensitive response or reaction (HR).

A brief account of these follows:

(i) Pathogenesis-Related (PR) Proteins:

These are products of defense-related genes that are activated by microbial infection and include hydrolytic enzymes such as:

(i) Proteinase inhibitors which inhibit activities of proteolytic enzymes secreted by the pathogen, and

(ii) Lytic enzymes such as glucanases, chitinases, and other hydrolases that attack and degrade the cell walls of the pathogen.

(ii) Biosynthesis of Phytoalexins:

The microbial infection also activates genes that encode enzymes for the synthesis of phytoalexins. Phytoalexins are a chemical diverse group of secondary metabolites (chiefly isoflavonoids and sesquiterpenes) with strong antimicrobial activity. The isoflavonoids medicarpin from alfalfa and glyceolin from soybean and sesquiterpenes such as rishitin from tomato and potato and capsidiol from tobacco and pepper are well known examples of phytoalexins.

(iii) Changes in Composition and Physical Properties of Host Cell Walls:
In response to pathogen invasion, lignin, callose, suberin and some hydroxy-proline rich glycoproteins are synthesized and accumulated in host cell walls to strengthen the latter and physically blocking the spread of the invading pathogen.

(iv) Programmed Cell Death (PCD):
The hypersensitive response culminates in rapid death of cells around the infection site depriving pathogen of the nutrient supply and limiting its spread in host plant and leaving necrotic lesions (small regions of dead tissues) at the site of invasion. The rest of the plant however, remains unaffected. Recent researches have shown that the hypersensitive response is preceded by accumulation of nitric oxide (NO) and active oxygen species (including the superoxide anion O_2^-, hydrogen peroxide H_2O_2 and hydroxyl radical OH).
The production of active oxygen species (known as the oxidative burst) and nitric oxide (a secondary messenger in many signalling pathways in animals and plants) appears to be prerequisite for activation of hypersensitive response. Induction of PCD is prevented in absence of any of these two signals.

It is believed that a plasma membrane located NADPH dependent oxidase reduces the O_2 to produce superoxide anions. The latter in turn are converted into hydrogen peroxide and hydroxyl radicals. The active oxygen species especially the hydroxyl radicals may contribute to PCD as part of the hypersensitive response or these may act to kill the pathogen directly. A transient increase in cytosolic Ca^{2+} concentration is required for the activity of the enzyme NO synthase which converts the amino acid arginine into nitric oxide.

Consequent to pathogen infection, both oxidative burst and NO production are activated by a transient change in plasma-membrane permeability resulting in influx of H^+ and Ca^{2+} ions into the cell and efflux of K^+ and CI^- ions.

Mechanism of Recognition of the Potential Pathogen to Initiate Defence Response in Plants:

Not all plants are resistant to disease caused by pathogens. Researchers have shown that resistance of plants to microbial pathogens has an underlying genetic basis. The pathogens carry avr genes (virulence genes) while the host plants carry corresponding resistance genes called R genes. Disease occurs when the pathogen lacks avr genes or the host plant does not carry dominant R genes.

The avr genes are believed to encode enzymes for the production of specific substances called elicitors while R genes encode protein receptors that recognise and bind with these elicitors to initiate the hypersensitive response.

The elicitors (L., elicere, to entice) are substances originating from pathogens that include proteins, peptides, sterols, and polysaccharide fragments arising from cell walls of pathogen or outer membrane or a secretion process. Sometimes, polysaccharide fragments resulting from initial degradation of the host plant cell walls by pathogen may elicit a hypersensitive response.

The receptor proteins encoded by R genes are located on plasma-membrane and have a leucine- rich domain which is repeated inexactly many times in the amino-acid sequence. These domains may extend outer or inner to the plasma-membrane and bind with specific elicitors to recognise the pathogen. (R genes comprise one of the biggest gene families in plants).

Method # 2. Secondary Acquired Resistance (SAR):

The hypersensitive response described earlier is limited to near vicinity of the initial site of infection by pathogen. But, often the entire host plants develops increased resistance against pathogens over a period of time ranging from few hours to several days following initial infection at one site of the plant. This phenomenon wherein "a single encounter with the pathogen increases resistance to entire plant to future attacks by pathogens" is called as secondary acquired resistance (SAR) and is part of the plant's immune response.

SAR appears to result from increased levels of some secondary metabolites and other defense compounds such as chitinases and other hydrolytic enzymes. However, the mechanism of SAR is not clearly understood. One component of the signalling pathway is likely to be salicylic acid (SA) that is a benzoic acid derivative and a secondary metabolite. It has been shown in variety of plants that the infection by pathogen results in increased levels of SA in the zone of infection that establishes SAR in distant regions of the plant (Fig. 23.5).

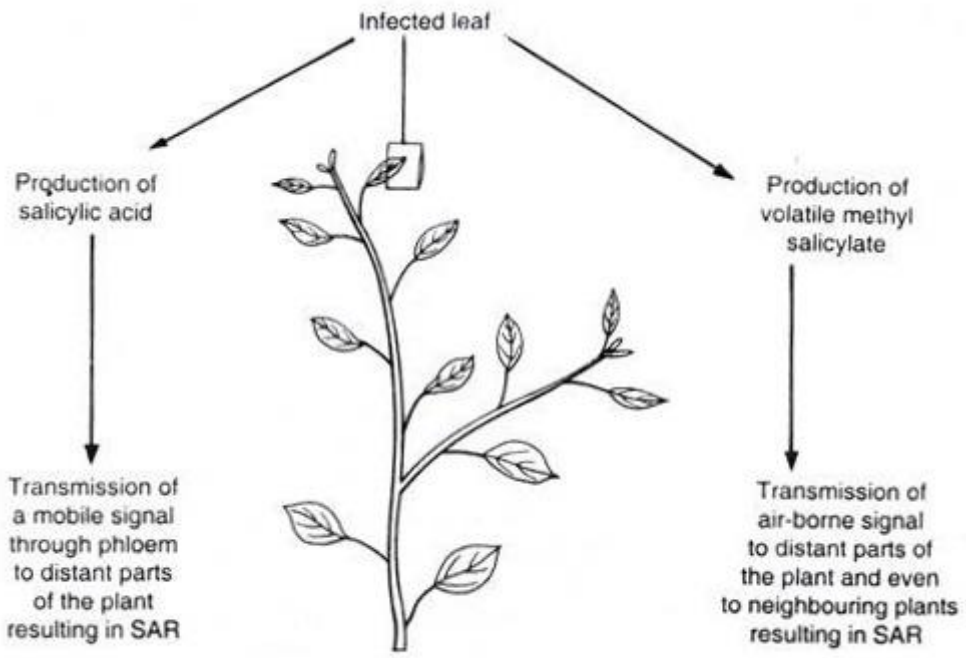


Fig. 23.5. Possible roles of Salicylic acid and methyl salicylate in SAR.

According to Van Bel and Gaupels (2004), the transmission of SAR signal from infection site to other parts of the plant is very rapid (3 cm/h) and possibly occurs through vascular tissue. Phloem is now considered to be the pathway of SAR signal.

Salicylic acid is not the mobile SAR signal. Maldonado et al (2002) have shown that in Arabidopsis, mutations in DIR1 gene (Defective in Induced Resistance 1 gene) inhibit SAR response. This gene is specifically expressed in phloem and encodes a lipid-transfer protein and it has been suggested that the long distance SAR signal might be a substance derived from a lipid.

Apart from phloem-mobile signals, the plant may develop SAR through air-borne signals. Salicylic acid may be converted into its methyl ester, methyl salicylate that is a moderately volatile substance. (Structures of Salicylic acid and methyl salicylate are given in Fig. 23.6). Methyl salicylate may function as volatile air-borne SAR-inducing signal that is transmitted to distant non-infected parts of the plant and even to non-infected neighbouring plants making them resistant to pathogens attack.

Fig. 23.6. Structures of salicylic acid and methyl salicylate.

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Weed Control Practices

The tandem technique of soil-tilling and herbicide application is an example of how farmers control weeds in their farms.

Generally, they till their soil before planting to reduce the number of weeds present in the field. Then they apply broad-spectrum or non-selective herbicides (one that can kill all plants) to further reduce weed growth just before their crop germinates. This is to prevent their crops from being killed together with the weeds. Weeds that emerge during the growing season are controlled using narrow-spectrum or selective herbicides. Unfortunately, weeds of different types emerge in the field, and therefore, farmers have to use several types of narrow-spectrum herbicides to control them. This weed control method can be very costly and can harm the environment.

Researchers postulated that weed management could be simplified by spraying a single broad-spectrum herbicide over the field anytime during the growing season.

Development of Glyphosate and Glufosinate Herbicide Tolerant Plants

Herbicide-tolerant (HT) crops offer farmers a vital tool in fighting weeds and are compatible with no-till methods, which help preserve topsoil. They give farmers the flexibility to apply herbicides only when needed, to control total input of herbicides and to use herbicides with preferred environmental characteristics.

Technology Background

How do these herbicides work?

These herbicides target key enzymes in the plant metabolic pathway, which disrupt plant food production and eventually kill it. So how do plants elicit tolerance to herbicides? Some may have acquired the trait through selection or mutation; or more recently, plants may be modified through genetic engineering.

Why develop HT crops?

What is new is the ability to create a degree of tolerance to broad-spectrum herbicides - in particular glyphosate and glufosinate - which will control most other green plants. These two herbicides are useful for weed control and have minimal direct impact on animal life, and are not persistent. They are highly effective and among the safest of agrochemicals to use. Unfortunately, they are equally effective against crop plants.

How do Glyphosate and Glufosinate HT crops work?

1. **Glyphosate-tolerant crops**

   Glyphosate herbicide kills plants by blocking the EPSPS enzyme, an enzyme involved in the biosynthesis of aromatic amino acids, vitamins and many secondary plant metabolites. There are several ways by which crops can be modified to be glyphosate-tolerant. One strategy is to incorporate a soil bacterium gene that produces a glyphosate-tolerant form of EPSPS. Another way is to incorporate a different soil bacterium gene that produces a glyphosate degrading enzyme.

2. **Glufosinate-tolerant crops**

   Glufosinate herbicides contain the active ingredient phosphinothricin, which kills plants by blocking the enzyme responsible for nitrogen metabolism and for detoxifying ammonia, a by-product of plant metabolism. Crops modified to tolerate glufosinate contain a bacterial gene that produces an enzyme that detoxifies phosphonothricin and prevents it from doing damage.

Other methods by which crops are genetically modified to survive exposure to herbicides including: 1) producing a new protein that detoxifies the herbicide; 2) modifying the herbicide's target protein so that it will not be affected by the herbicide; or 3) producing physical or physiological barriers preventing the entry of the herbicide into the plant. The first two approaches are the most common ways scientists develop herbicide tolerant crops.

Safety Aspects of Herbicide Tolerance Technology

Toxicity and Allergenicity

Government regulatory agencies in several countries have ruled that crops possessing herbicide-tolerant conferring proteins do not pose any other environmental and health risks as compared to their non-GM counterparts.

Introduced proteins are assessed for potential toxic and allergenic activity in accordance with guidelines developed by relevant international organizations. They are from sources with no history of allergenicity or toxicity; they do not resemble known toxins or allergens; and they have functions, which are well understood.

Effects on the Plants

The expression of these proteins does not damage the plant's growth nor result in poorer agronomic performance compared to parental crops. Except for expression of an additional enzyme for herbicide tolerance or the alteration of an already existing enzyme, no other metabolic changes occur in the plant.

Persistence or invasiveness of crops

A major environmental concern associated with herbicide-tolerant crops is their potential to create new weeds through outcrossing with wild relatives or simply by persisting in the wild themselves. This potential, however, is assessed prior to introduction and is also monitored after the crop is planted. The current scientific evidence indicates that, in the absence of herbicide applications, GM herbicide-tolerant crops are no more likely to be invasive in agricultural fields or in natural habitats than their non-GM counterparts (Dale et al., 2002).

The herbicide-tolerant crops currently in the market show little evidence of enhanced persistence or invasiveness.

Advantage of Herbicide Tolerant Crops

- Excellent weed control and hence higher crop yields;
- Flexibility – possible to control weeds later in the plant's growth;
- Reduced numbers of sprays in a season;
- Reduced fuel use (because of less spraying);
- Reduced soil compaction (because of less need to go on the land to spray);
- Use of low toxicity compounds which do not remain active in the soil; and
- The ability to use no-till or conservation-till systems, with consequent benefits to soil structure and organisms (Felsot, 2000).

A study conducted by the American Soybean Association (ASA) on tillage frequency on soybean farms showed that significant numbers of farmers adopted the "no-tillage" or "reduced tillage" practice after planting herbicide-tolerant soybean varieties. This simple weed management approach saved over 234 million gallons of fuel and left 247 million tons of irreplaceable topsoil undisturbed.

Current Status of Herbicide Tolerance

From 1996 to 2013, herbicide- tolerant crops consistently occupied the largest planting area of biotech crops. In 2013 alone, herbicide tolerant crops occupied 99.4 million hectares or 57% of the 175.2 million hectares of biotech crops planted globally. The most common are the

glyphosate and glufosinate tolerant varieties. The following table shows countries that have approved major HT crops for food, feed and/or cultivation.

Crop	**Countries**
Alfalfa	Australia, Canada, Japan, Mexico, New Zealand, Philippines, Singapore, South Korea, United States of America (USA)
Argentine Canola	Australia, Canada, Chile, China, European Union (EU), Japan, Mexico, New Zealand, Philippines, Singapore, South Africa, South Korea, USA
Carnation	Malaysia
Chicory	USA
Cotton	Argentina, Australia, Brazil, Canada, China, Colombia, Costa Rica, EU, Japan, Mexico, New Zealand, Paraguay, Philippines, Singapore, South Africa, South Korea, USA
Flax, Linseed	Canada; Colombia; USA
Maize	Argentina, Australia, Brazil, Canada, China, Colombia, EU, Honduras, Indonesia, Japan, Malaysia, Mexico, New Zealand, Panama, Paraguay, Philippines, Russian Federation, Singapore, South Africa, South Korea, Switzerland, Taiwan, Thailand, Turkey, USA, Uruguay
Polish Canola	Canada
Potato	Australia, Canada, Japan, Mexico, New Zealand, Philippines, South Korea, USA
Rice	Australia, Canada, Colombia, Honduras, Mexico, New Zealand, Philippines, Russian Federation, South Africa, USA
Soybean	Argentina, Australia, Bolivia, Brazil, Canada, Chile, China, Colombia, Costa Rica, EU, India, Indonesia, Japan, Malaysia, Mexico, New Zealand, Paraguay, Philippines, Russian Federation, Singapore, South Africa, South Korea, Switzerland, Taiwan, Thailand, Turkey, USA, Uruguay
Sugarbeet	Australia, Canada, China, Colombia, EU, Japan, Mexico, New Zealand, Philippines, Russian Federation, Singapore, South Korea, USA
Wheat	Australia, Colombia, New Zealand, USA

Source: ISAAA's GM Approval Database. http://www.isaaa.org/gmapprovaldatabase/.

A literature review conducted by the Council for Agricultural Science and Technology concluded that the environment benefits from the use of HT crops. In the US, for example, no-till soybean acreage has increased by 35% since the introduction of HT soybean. A similar trend is observed in Argentina where soybean fields are 98% planted with HT varieties. The CAST paper entitled “Comparative Environmental Impacts of Biotechnology-derived and Traditional Soybean, Corn and Cotton Crops” is available at http://www.cast-science.org.

For the first 17 years of commercialization (1996-2012), benefits from herbicide tolerant crops are valued at US$ 47.7 billion, 41% of global biotech crop value of US$ 116.9 billion, and for 2012 alone at US$ 6.6 billion or 35% of global value of US$ 18.7 billion.

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http://isaaa.org/resources/publications/pocketk/10/default.asp

Plant derived vaccines

Vaccine

- A vaccine is a biological preparation that improves immunity to a particular disease.
- A vaccine typically contains an agent that resembles a disease-causing microorganism, and is often made from weakened or killed forms of the microbe, its toxins or one of its surface proteins.
- The agent stimulates the body's immune system to recognize the agent as foreign, destroy it, and "remember" it, so that the immune system can more easily recognize and destroy any of these microorganisms that it later encounters.
- Vaccination is the most effective and cost-efficient method for fighting human and animal diseases, preventing the spread of infectious diseases, completely eradicating some diseases and even helping to prevent cancer and autoimmune disorders.
- There are now more then 25 vaccines licensed for use in humans with many more in the development pipeline.
- An effective vaccine should be inexpensive, confer protective immunity to the vaccine with few side effects and safe, stable and easy to administer.

Different methods of vaccine production

- **Traditional vaccines:**

Traditional vaccines are live, killed or otherwise attenuated/modified pathogens (e.g., influenza vaccines produced in specific pathogen free-eggs). However, they have been increasingly supplanted by recombinant subunit vaccines produced in genetically modified cells because they offer increased safety, less antigenic competition, the ability to target vaccines to specific sites and the ability to differentiate between infected and vaccinated animals.

- **Production systems for recombinant subunit vaccines:**

Recombinant subunit vaccines currently on the market are produced in bacteria, yeast or insect cells and mammalian cells.

- Bacterial systems for vaccine production cannot produce more complex proteins that folds correctly and undergo forms of post-translational modifications (e.g. N-glycosylation). Furthermore, the presence of endotoxins and other pyrogens has limited their use.

- Since yeast cells are eukaryotes, they can fold and assemble complex recombinant proteins and carry out N-glycosylation however, the glycan structures often differ to those found in mammals.
- Insect cells culture medium is much less expensive and tend not to harbor mammalian pathogens. However, insect cells have limited scalability and major differences in glycan structures could raise challenges for the production of some recombinant proteins.
- In case of mammalian cells factors such as the cost of infrastructure and consumables, and the need for extensive product validation to prove that the final product is pathogen-free and does not contain oncogenic agents make this platform commercially unfeasible for vaccines required on a large scale.

Plants as a platform for the production of cost-effective proteins

- Plants have been used to produce over 200 medically relevant proteins and their many benefits now make them a serious competitor to mammalian cells for biopharmaceutical production.
- In terms of vaccine production, plants share the advantages of other eukaryotic systems but lack their principle disadvantages in terms of cost, safety and scalability.
- Plant-derived vaccines can be produced either stably by nuclear and chloroplast transformation or transiently by tobacco mosaic virus (TMV) based expression.

Advantages of plant-derived vaccines

- With plants, there is no need to build and run expensive fermenters, hire skilled workers and pay for expensive culture media.
- Very high yields of recombinant proteins can be achieved by chloroplast transformation. In two recent reports, approximately 70% and 72% of total soluble protein (TSP) has been obtained by plastid transformation, respectively.
- Growth of plants can be scaled up according to the required amount of protein.
- Plants tend not to harbor human pathogens and any colonizing bacteria or animal-derived material can be removed using appropriate sanitary measures before processing.
- Transgenic plants can be grown at the site where the vaccine is needed. This advantage can save the costs related to transportation and cold storage.
- Plant-derived vaccines have the potential to be used as oral vaccine, thus evading the costs related to sterile needles and trained medical staff.
- Plants-derived vaccines are likely to be more stable. Proteins expressed in certain plant tissues (e.g., cereal seeds) remain stable for years at ambient temperatures without loss of activity.

- Plant-derived vaccines survive in the stomach through bioencapsulation, which allows gradual release and, in some, cases this makes the vaccine more efficacious than the same subunit delivered through the parenteral route.

Vector	Cost to produce	Length of time to produce	Glycosylation	Risk of pathogen contamination	Cost to store
Bacteria	Low	Short	No	Medium	Moderate
Mammalian cells	High	Long	Yes	High	Expensive
Transgenic plants	Low-medium	Long	Yes(some differences)	Low	Low

Plant-derived vaccines: brief history

- The first demonstration of expression of a vaccine antigen within plants occurred in 1990 when Curtiss and Cardineau expressed the *Streptococcus mutans* surface protein antigen A (SpaA) in tobacco.
- This demonstration was closely followed by plant expression of the hepatitis B surface antigen (HbsAg), the *E. coli* heat–labile enterotoxin responsible for diarrhoea, the Norwalk virus capsid protein and the rabies virus glycoprotein.
- Proteins produced in these plants induced synthesis of antigen specific mucosal IgA and serum IgG when delivered orally to mice and humans.
- The latest landmark in the development of pharmaceutical-producing plants sees a tomato-derived vaccine against cholera and hepatitis C
- Researchers have combined genetic sequences of these two pathogens and introduced them into plants. The tomato plants then produce key proteins of both pathogens.
- One of the advantages of the tomato-derived vaccine is that it is easily stored in the seed of the tomatoes themselves, according to lead researcher.
- Various vaccine antigens against different human diseases have been successfully expressed in chloroplast. However, none of the chloroplast-derived vaccine candidate has yet entered into clinical trials.
- So far, few plant-derived vaccines against human diseases, expressed either by nuclear transformation or TMV based expression, have shown promising results in phase I and II clinical trials upon oral delivery.

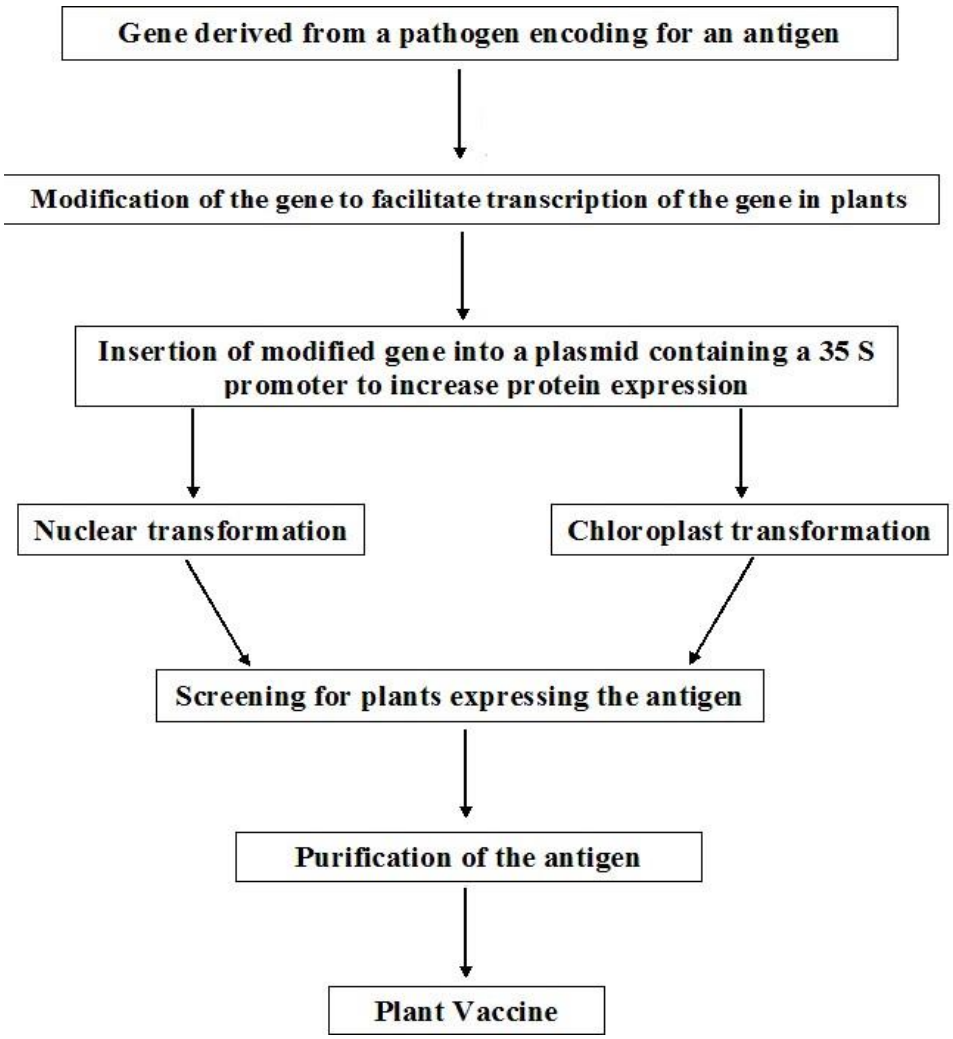
Diversity of plant-based systems

- To date many plant species have been used for vaccine production.

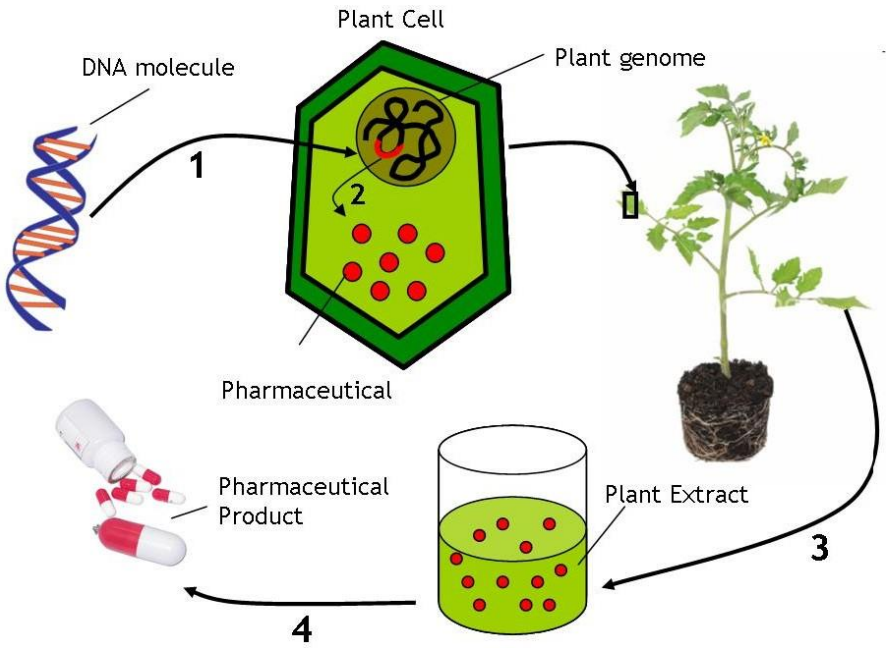
- Early studies used tobacco and potato but now tomato, banana, corn, carrot, lettuce and others are being used for this purpose.
- The choice of the plant species (and tissue in which the protein accumulates) is important and is usually determined through how the vaccine is to be applied in the future.

Procedure of plant-derived vaccines

- A DNA molecule carrying the genetic information for a pharmaceutical substance is introduced into the plant genome.
- This process is called transformation. The genes can be incorporated permanently (stable transformation) or for a short period of time (transient transformation). The transformed plant acts as a bioreactor producing large quantities of the pharmaceutical using its protein making machinery
- Through industrial processing, the pharmaceutically active substance is extracted from the plant and made into in a formulated product, for example a pill.



Flow diagram of the general procedures used to produce plant made vaccines



Strategies for vaccine production in plants

- **Transgenic plants:**

The most widely used strategy for vaccine production in plants is the nuclear transgenic system, in which the antigen transgenes are transferred to the plant nuclear genome.

- **Transplastomic plants:**

Instead of introducing transgenes into the nuclear genome, they can be targeted to the chloroplast genome using particle bombardment or other physical DNA delivery techniques, ensuring that the transgene is embedded in a chloroplast DNA homology region.

- **Plant viral expression system:**

This expression system is based on infection of a plant by a plant virus, which is competent to independently replicate, transcribe, and translate so as to produce many copies of a recombinant protein introduced into the plant viral genome.

Antigens produced in transgenic plants

Recombinant protein (vaccine)	Transgenic plant	Protection against
Rabies glycoprotein	Tomato	Rabies virus
Foot and mouth virus (VPI)	Arabidopsis	Foot and mouth virus
Herpes virus B surface antigen	Tobacco	Herpes simplex virus
Cholera toxin B subunit	Potato	*Vibrio cholerae*
Human cytomegalovirus glycoprotein B	Tobacco	Human cytomegalovirus

Oral delivery, mucosal and systemic antibody responses

- Plant-derived vaccines have demonstrated the ability to induce both systemic and mucosal immune responses.
- Most infectious agents enter the body through mucosal membranes. Induction of mucosal immunity is best achieved by direct vaccine delivery to mucosal surfaces.
- While effective inducers of systemic immunity, vaccines delivered by injection are not efficient at inducing mucosal responses.
- The major obstacle to oral vaccination is the digestion of the antigenic protein in the stomach.
- Vaccines derived and delivered by plant cells have been shown to overcome this problem through the protective effect of the plant cell wall.
- Like liposomes and microcapsules, the plant cell wall allows gradual release of the antigen onto the vast surface area of the lower digestive tract.
- Further problems may be associated with poor immunogenicity or the induction of tolerance. Binding to a targeting molecule or carrier peptide, has been shown to overcome poor immunogenicity of orally delivered subunit vaccines.
- In specific circumstances, for example cancer therapy, injection of the drugs, after purification from the producing plant, may be preferred.

Targets for plant-derived proteins

Vaccines against infectious diseases:

- There is a large and fast growing list of protective antigens from microbial and viral pathogens that have been expressed by plants.
- The initial focus was upon human pathogens. However, today attention has also spread to animal pathogens (e.g. Newcastle and foot and mouth disease).

Vaccines against autoimmune diseases:

- Transgenic plants expressing autoantigens are being produced in attempt to cure diseases in which the immune system recognises the body's own proteins as foreign. The diseases include arthritis, multiple sclerosis, myasthenia gravis, and type I diabetes.
- The rational is that an appropriate oral dose of a plant-derived autoantigen will inhibit the development of the autoimmune disease.

Table 2. Transient production of antigens in plants after infection with plant viruses expressing a recombinant gene

Protein	Plant	Carrier	Refs
Influenza antigen	Tobacco	TMV	28
Murine zona pellucida antigen	Tobacco	TMV	28
Rabies antigen	Spinach	AIMV	29
HIV-1 antigen	Tobacco	AIMV	30
Mink enteritis virus antigen	Black eyed bean	CPMV	31
Colon cancer antigen	Tobacco	TMV	32

AIMV, alfalfa mosaic virus; TMV, tobacco mosaic virus; CPMV, cowpea mosaic virus.

Vaccines against human tumours:

- Particular proteins have been shown to over-express on the cell surface of many tumours, including melanom and breast cancer. Naturally acquired, actively induced or passively administered antibodies against these antigens have been able, in some cases, to eliminate circulating tumour cells and micrometastasis. However, cancer vaccine development is complicated due to the tumour antigens also being auto-antigens.

Advantages of plant-derived vaccines

- Cost effective.
- Easy to administer.
- Easy to store.
- Acceptable to poor developing country.
- Safe
- Activate both mucosal and systemic immunity.
- Heat stable.
- Do not required cold chain maintenance.
- No fear of contamination.

Disadvantages

- One of the fears is that GM-pollen may outcross with sexually compatible plants (related crops or weeds) and affect biodiversity.
- Producing vaccines in plants also has a drawback because of the associated contamination risk for food crop production.
- A soya field was found to be contaminated with transgenic maize producing the pharmaceutically-active substance trypsin causing the complete harvest of 13,500 tons of soya beans to be destroyed. Hence, research is moving towards non-foods such as tobacco.
- There is also the risk that the pharma plants could be eaten by animals.
- The active substances could enter the groundwater and lead to harmful effects.
- Antibiotic resistance marker genes can spread from GM food to pathogenic bacteria.
- If a vaccine was consumed inadvertently, it could lead to desensitisation so that vaccinations would eventually cease to work.

References

http://www.bioethics.iastate.edu/retreat_2003/powerpoint/wang.ppt

Edible vaccine

- Edible Vaccine involves introduction of selected desired genes into plant and then inducing these altered plants to manufacture the altered protein.

How it acts?

- Antigen in transgenic plant
  > Ingestion
  >Delivered by bioencapsulation
  >Taken up by Mcell
  > Pass on to the Macrophage
  >IgG, IgE responses
  > Local IgA response & Memory cells
  > Neutralize the attack by the real infectious agent.

Advantage and Disadvantage of different plants

Plant	Advantages	Disadvantages
Potato	• Easily transformed. • Easily propagated. • Stored for long periods without refrigeration.	• Need cooking which denature antigen.
Banana	• Do not need cooking. • Protein not destroyed even after cooking. • Inexpensive. • Grown widely in developing countries.	• Trees take 2-3 to mature years. • Spoils rapidly after ripening.
Rice	• Commonly used in baby food. • High expression of antigen.	• Grows slowly. • Requires glasshouse condition.
Tomato	• Grow quickly. • Cultivate broadly. • High content Vitamin-A may boost immune response.	• Spoils readily.

Clinical trials on:

- **ETEC**

11 Volunteers were feed raw transgenic Potatoes expressing LT-B.

10 (95%) of these individuals developed neutralizing antibodies and 6 (55%) develop mucosal response.

- **Norwalk Virus**

20 people fed with transgenic potato .

19 (95%)of them expressing Norwalk virus antigen showed sero conversion.

- **Hepatitis B**

First human trials of potato-based vaccine against Hepatitis B have reported encouraging results.

The amount of HBsAg needed for one dose could be achieved in a single potato.

Levels of specific antibodies significantly exceeded the protective level of 10mIU/mL in human.

Advantages of Edible Vaccine

- Cost effective.
- Easy to administer.
- Easy to store.
- Acceptable to poor developing country.
- Fail safe
- Activate both mucosal and systemic immunity.
- Heat stable.
- Do not required cold chain maintenance.
- No fear of contamination.

Future of Edible Vaccine

- Resistance to GM foods may affect future of Edible Vaccine.

Limitations

- Transgenic contamination can occur.
- Antibiotic resistance marker genes can spread from GM food to pathogenic Bacteria.
- Difficulty in dose maintenance.

Edible plant derived vaccine may lead to a future of safer and more effective immunization.

Plantibodies

A **plantibody** is an antibody that is produced by plants that have been genetically engineered with animal DNA. An antibody (also known as an immunoglobulin) is a complex protein within the body that recognizes antigens on viruses and other dangerous compounds in order to alert the immune system that there are pathogens within the body.[1] The transgenic plants become transformed with the DNA and produce antibodies that are similar to those inserted. The term plantibody and the concept are trademarked by the company Biolex.

A plantibody is produced by insertion of antibodies into a transgenic plant. The plantibodies are then modified by intrinsic plant mechanisms (N-glycosylation). Plantibodies are purified through processes such as filtration, immunofluorescence, chromatography, and diafiltration. It is more cost effective to produce antibodies in transgenic plants than in transgenic animals.

Antibodies produced from plants (Smith & Glick, 2000)

- Functional antibodies can be produced from plants
- Targeted to intercellular space, chloroplast, seeds and tubers
- Benefit for topical immunotherapy
- Can be produced from plants in large quantities
- Plantibodies provides increased stability. e.g: Secretory Ig A
- Plants can assemble complex secretory antibodies
  - e.g: Construction of tobacco plants expressing 4 transgenes
- Quadruple transgenics efficiently assembled secretory immunoglobulins

Full-size monoclonal antibodies recently produced in transgenic plants

Plant	Antibody type (target)	Purpose
Tobacco	IgG (low molecular weight phosphonate ester)	Catalytic antibodies
Tobacco	IgG (nematode)	Plant pathogen resistance
Tobacco	sIgA/G (*Streptococcus mutans*)	Therapeutic (topical)
Soybean, rice	IgG (herpes simplex virus)	Therapeutic (topical)
Tobacco	IgG (colon cancer)	Therapeutic (systemic injection)
Alfalfa	IgG (human IgG)	Diagnostic
Tobacco	IgG (rabies virus)	Therapeutic: first IgG expressed in plant showing therapeutic activity (systemic injection)
Tobacco	IgG (hepatitis B virus)	Immunopurification of hepatitis B surface antigen
Tobacco	IgG (hepatitis B virus)	Therapeutic

Anti-rabies virus mAb

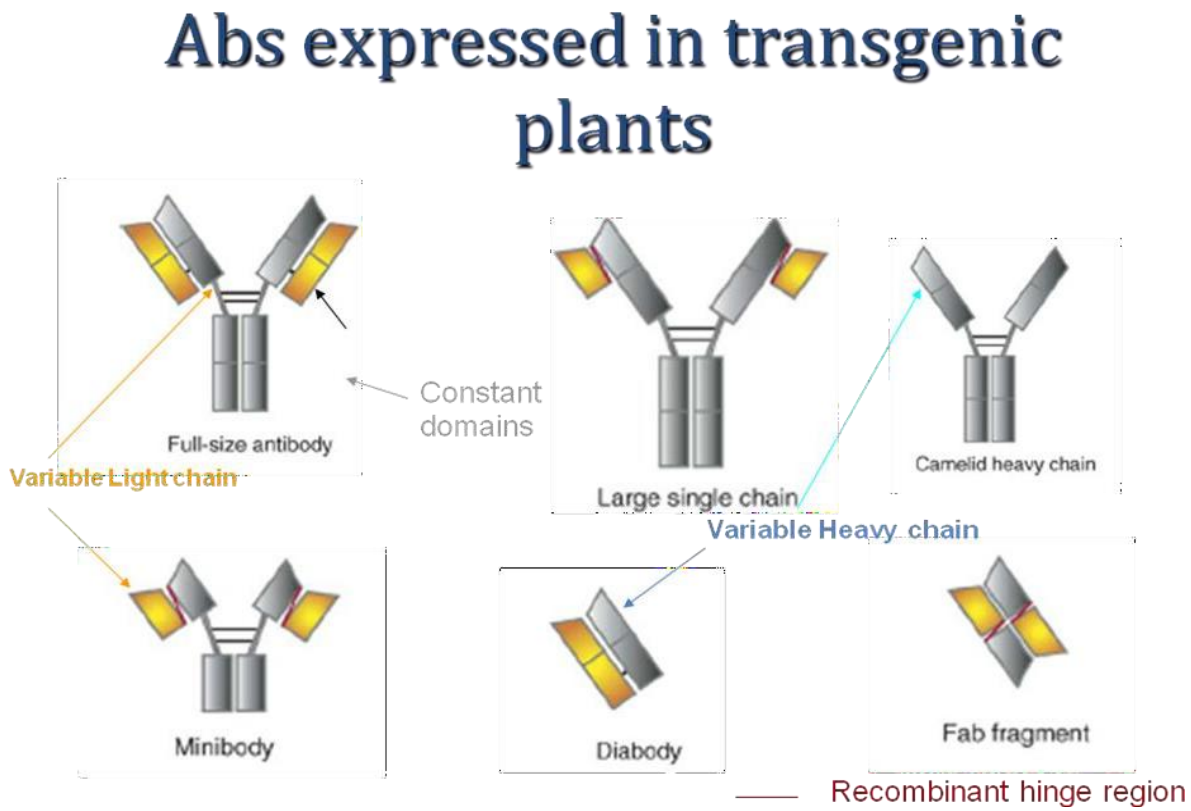
- After exposure treated with Ab

- Used to be made in horses
- First mAb made in transgenic plants
- 4 genes – 2 H, 2 L
- Transgenic plant for each one and crossing plants
- Later used single binary vector with two promoters

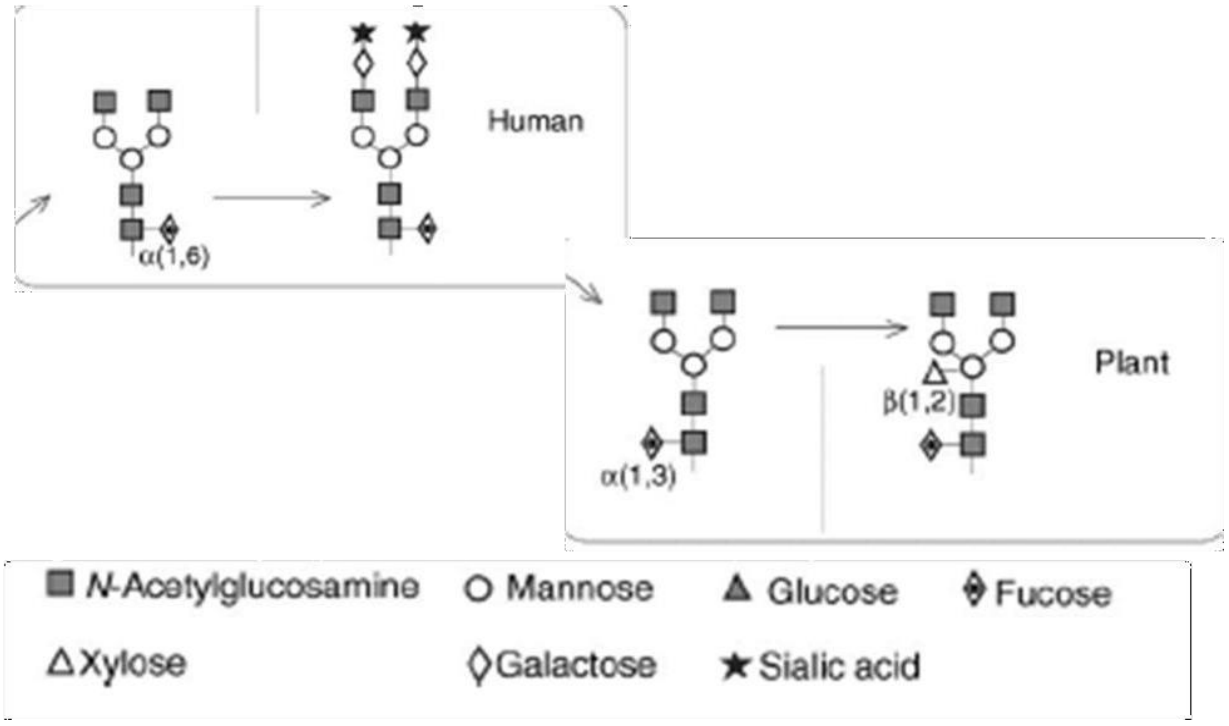
Functional Abs

- Need to be properly folded and assembled
- Need disulfide bond formation and glycosylation
- Down stream processing:
    - Purification of Ab mostly with Protein A or G
- Glycosylation is different in plants
    - β 1,2 Xylose and α 1,3 fucose
- Retain in ER, only mannose is attached

  Shorter half-life of Ab



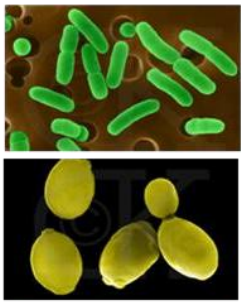
Glycosylation in Golgi



Production Costs for Antibodies

Production costs	cost in $ / gram
hybridomas	1000
transgenic animals	100
transgenic plants	10

Source: Daniell et al. (2001) TIPS 6, 219-226



E. coli & yeast

Tr. animals and animal cells

Transgenic plants

Antibodies: a compelling success story

- high specificity: *in vitro* and *in vivo* diagnostics
- low toxicity: therapeutic applications
- high drug approval rates (24 approved mAbs)
- major products in biotechnology (~240 in clinical trials)
- inherently stable human proteins
- injectable, topical and oral applications
- applicable for chronic conditions
- potential long-lasting benefits

Comparison of Mammalian and Plant-produced Antibodies

- peptide sequence: identical
- correct cleavage of Ig-derived signal peptides
- kinetics & affinity: identical
- stability in seeds > 30 months
- antibody types: plant system more versatile (sIgA)
- post-translational processing: different
- core glycan identical, terminal sugar different plus xylose & fucose
- antigenicity & clearance: apparently identical (shorter half-life)

Advantages of Plantibodies

- Produced in containment
- Controlled conditions
- Correctly folded, soluble proteins
- High levels of expression
- Cost effective for large scale production
- Safety issues

Disadvantages of Plantibodies

- Sporadic transgene silencing
- Glycolsylation patterns
- Inefficient expression
- Environmental contamination

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Insect resistance plants

Definitions of an insect-resistant plant are many and varied. In the broadest sense, plant resistance is defined as "the consequence of heritable plant qualities that result in a plant being relatively less damaged than a plant without the qualities." In practical agricultural terms, an insect-resistant crop cultivar is one that yields more than a susceptible cultivar when confronted with insect pest invasion. Resistance of plants is relative and is based on comparison with plants lacking the resistance characters, i.e., susceptible plants.

Tools of molecular biology and genetic engineering have provided humankind with unprecedented power to manipulate and develop novel crop genotypes towards a safe and sustainable agriculture in the 21st century. Technologies and chemical inputs that have proven harmful to human health and environment need to be replaced with safer alternatives to manage insect pests in agricultural ecosystems.

Many insecticidal proteins and molecules are available in nature which are effective against agriculturally important pests but are innocuous to mammals, beneficial insects and other organisms. Insecticidal proteins present in Bacillus thuringiensis (Bt), which have shown efficacy as spray formulations in agriculture over the past five decades, have been expressed in many crop species with positive results. Large scale cultivation of Bt-crops raises concerns about the possible development of resistant insects. Many strategies have been formulated to prevent/delay the development of resistance.These strategies have to be given serious consideration in India where the first Bt-crop containing resistance to insect pests, particularly Helicoverpa armigera, has been released for commercial cultivation in the farmers' fields.

In addition to Bt, proteinase inhibitors present in several plant species offer a good source of resistance to insect pests. A combination of proteinase inhibitors has been suggested as a viable alternative to Bt to manage insects such as H.armigera. In recent years, several novel insecticidal proteins have been discovered in bacteria such as Photorhabdus luminescens. The judicious expression of multiple insecticidal proteins that differ in their mechanisms of toxicity will provide formidable barriers for insects to develop resistance. Finally, deployment of integrated pest management (IPM) strategies during the cultivation of transgenic crops will ensure durable insect resistance.

Expression of Insecticidal proteins of Bacillus thuringiensis

Bacillus thuringiensis (Bt) is a Gram-positive, aerobic, sporulating bacterium which synthesizes crystalline proteins during sporulation. These crystalline proteins are highly insecticidal at very low concentrations

Expression of Vegetative insecticidal proteins of Bt

Research efforts in the past five years have led to the discovery of novel insecticidal proteins which are produced by certain isolates of B. thuringiensis. These proteins unlike well characterized crystal proteins are produced during vegetative growth of cells and are secreted into the growth medium.

Expression of other insecticidal proteins from bacteria, plants and animals

Proteinase inhibitors
Plant lectins
α -amylase inhibitors
Insect chitinases
Plant metabolic enzymes
Insecticidal viruses
Genes from bacteria other than Bt
Novel genes of plant origin

Stress tolerance plants

Abiotic stress is one of the primary causes of crop losses worldwide but crop plants are affected by a variety of abiotic stresses. Abiotic stresses includes drought, salinity, heat, cold, flooding, and ultraviolet radiation etc. Currently, there are no economically viable technological means to facilitate crop production under stress conditions using breeding methods. However, the development of crop plants tolerant to drought stress is considered a promising approach, which may help satisfy growing food demands from both developing and under-developed countries. By contrast, improvement of stress tolerance by genetic engineering overcomes the bottlenecks of plant breeding methods. Transgenic approaches can be used in combination with conventional breeding strategies to create crops with enhanced drought tolerance. The current genetic engineering strategies rely on the transfer to the targetplant of one or several genes that are either involved in signaling and/or regulatory pathways, or that encode enzymes present in

pathways leading to the synthesis of functional and structural protectants, such as osmolytes and antioxidants, or pathways that encode stress tolerance-conferring proteins.

Plants have evolved various mechanisms to cope with stress conditions and these include the shifts in the physiology of the plant and the expression of stress-associated genes, leading to the formation of a wide variety of low molecular mass metabolites collectively known as compatible solutes such as proline, glycine betaine, sugars such as sucrose, trehalose and fructans and sugar alcohols like sorbitol, mannitol, ononitol, pinitol and polyols These osmolytes are uniformly neutral with respect to the perturbation of cellular functions even when present at high concentrations. Accumulation of these molecules either actively or passively helps plants to retain water within cells and protects cellular compartments from injury caused by dehydration or maintains turgor pressure during water stress. Moreover, these molecules stabilize the structure and function of certain macromolecules, signaling functions or induction of adaptive pathways and scavenge reactive oxygen species. However, the molecular and cellular interactions of these solutes are not completely understood.

Identifying the mechanisms developed and deployed by plants to counteract abiotic stresses and maintain their growth and survival under harsh conditions thus holds great significance. Recent investigations have shown that phytohormones, including the classical auxins, cytokinins, ethylene, and gibberellins, and newer members including brassinosteroids, jasmonates, and strigolactones may prove to be important metabolic engineering targets for producing abiotic stress-tolerant crop plants.

Strategies for Improving Crops Against Stresses

- Use of Naturally Stress Tolerant Plants
- Selection and Breeding for Drought Tolerance
- Molecular Breeding
- Transgenic Approach

Plant as bioreactors

Plant as bioreactors refer to the use of transgenic plants and cell cultures of plants to make unlimited quantities of commercially important substances like recombinant proteins including antibodies and vaccines using biotechnology oriented techniques.

Most of the research has been directed towards using plant bioreactors to make the following

- Therapeutic proteins
- Edible vaccines
- Antibodies for immunotherapy

Using genetic engineering, cereal plants, fruits plants, legumes and vegetable plants have the capacity to become low cost bioreactors to make molecules that in the normal scheme of things would not have been available from plants. Human growth hormone was the first drug that was produced using plant bioreactors, in this case from the transgenic tobacco.

Here we discuss the different plant bioreactor expression systems especially with reference to where the protein compartmentalizes in these bioreactors.

Seed-based plant bioreactors

Plant seeds accumulate large amount of proteins during their development stage and therefore plant bioreactors based on seed platforms are reckoned as suitable for storing recombinant proteins.

An example is the successful expression of the human lysosomal enzyme alpha-L-iduronidase in Arabidopsis thaliana seeds. In seed bioreactors the expression of recombinant proteins is controlled using seed specific promoters like for example in maize globulin-1, and in rice glutelin promoter Gt-1. The advantage of these systems is that, proteins do not degrade at ambient temperature and are stable for long term storage.

However factors such as specificity of expression and subcellular storage environment would decide how specifically seeds could be used for producing desired molecules.

Seed Protein Storage Vacuole Bioreactors

In seed bioreactors, protein storage vacuoles comprising the sub compartments namely, matrix, globoid and crystalloid are dominant compartments for storing recombinant proteins. The matrix is suitable for soluble storage proteins, globoid for hydrolytic enzyme, and crystalloid for BP-80 TMD and the CT of alpha-tonoplast intrinsic protein sequences.

Seed Oil Body Bioreactors

Seed oil body is encircled by the protein oleosin which are ideal carriers for heterologous protein production and also provide a recognition signal for lipase binding during oil mobilization in seedlings. Seed oil body is bioreactors that can store large amount of macromolecules.

An example is the expression of fusion protein containing oleosin and the β-glucuronidase in seed oil body. Another example is the manufacture of the anticoagulant hirudin in the oil body of seeds Brassica napus and Brassica carinata.

Plant Suspension Cultures

They are used to express recombinant proteins, secondary metabolites and antibodies transported to subcellular organelles. For example, is the expression of 80-kDa human lysosomal protein (controlled by 35S CaMV and a signal peptide) in transgenic tobacco BY-2 cells culture media.

Hairy Root System Bioreactor

The Hairy Root System with its rhizosecretion is due to infection of the soil bacterium-Agrobaterium rhizogenes. It offers extreme biosynthetic stability and is suitable for making biopharmaceuticals as for example scopolamine in Hyoscyamus muticus L. hairy root culture

Chloroplast bioreactor

Insulin, interferons and other biopharmaceutical proteins can be made using Chloroplast bioreactor. One method is foreign genes are inserted into nuclear chromosomes and with peptides target expressed proteins into chloroplast. An example is the high yield in the expression of human serum albumin protein in chloroplast.

Comparative study of plant bioreactor systems

Cost wise, seed, hairy root, cultured cell suspension, oil body and chloroplast bioreactor systems are on the cheaper side to set-up and in terms of product stability seed and oil body systems offer the best prospects. In terms of scale-up capacity seed, oil body and chloroplast bioreactor systems are most suitable.

Advantages and Disadvantages

Generally the practice has been to use cloned animal cells to make antibodies for use as drugs. But there is always the remote chance of unwanted allergic reactions due to

antibodies of animal origin, and that apart, contamination of the antibody product due to proteins and viruses of animal origin is a distinct possibility. Such problems do not arise when plants are used to make antibodies because plants do not generally serve as hosts for human and animal pathogens.

Making recombinant proteins in transgenic plants is relatively cheap as compared to the cost of running fermentors although extraction and purification processes have to be efficient. For example these proteins are expressed in the plant seeds which all look similar and the recombinant protein has to be cleaved enzymatically or by other means. But these seeds can be stored and processed when required unlike animal cell production methods which require immediate purification.

Conclusion

More and more uses of plant bioreactors are coming up these days. For example, plant bioreactors are being investigated for making enzymes suitable for use in feed additives and in food. Another use of plants is to make genetically engineered plants that can produce seeds which can function as a delivery mechanism for various industrial enzymes.

As you can see these processes go far beyond the application of biotechnology in traditional agriculture, and so today, transgenic plants can produce on a mass scale proteins for agricultural, veterinary and pharmaceutical use. These processes are mainly carried out by cost conscious biopharma and enzyme companies.

Recombinant & subunit vaccine

Plant based vaccines are subunit vaccines in which the antigen of interest is expressed in plant tissues.

Molecular basis of photosynthesis

What is photosynthesis?

Photosynthesis is a biological process whereby the Sun’s energy is captured and stored by a series of events that convert the pure energy of light into the biochemical energy needed to power life. This remarkable process provides the foundation for essentially all life and has over geologic time profoundly altered the Earth itself. It provides all our food and most of our energy resources.

Perhaps the best way to appreciate the importance of photosynthesis is to examine the consequences of its absence. The catastrophic event that caused the extinction of the

dinosaurs and most other species 65 million years ago almost certainly exerted its major effect not from the force of the comet or asteroid impact itself, but from the massive quantities of dust ejected into the atmosphere. This dust blocked out the Sun and effectively shut down photosynthesis all over the Earth for a period of months or years. Even this relatively short interruption of photosynthesis, miniscule on the geological time scale, had catastrophic effects on the biosphere. Photosynthesis means literally “synthesis with light.” As such, it might be construed to include any process that involved synthesis of a new species under the action of light. However, that very broad definition might include a number of unrelated processes that we do not wish to include, so we will adopt a somewhat narrower definition of photosynthesis:

Photosynthesis is divided into two sets of reactions: the light-dependent (light) reactions and the light-independent (dark) reactions. As their names imply, the first set depends directly on light, whereas the second set does not. Nevertheless, even the dark reactions will cease if the plants are deprived of light for too long because they rely on the products of the light reactions.

The light reactions, which convert the energy in light into chemical energy, take place within the thylakoid membranes of the chloroplasts, whereas the dark reactions, which use that chemical energy to fix CO_2 into organic molecules, take place in the stroma of the chloroplast. In the light reactions, the energy of light is used to "split water," stripping a pair of electrons from it (and causing the two hydrogens to be lost), thus generating molecular oxygen. The energy in light is transferred to these electrons, and is then used to generate adenosine triphosphate (ATP) and the electron carrier NADPH. These two products carry the energy and electrons generated in the light reactions to the stroma, where they are used by the dark reactions to synthesize sugars from CO_2 .

Within the chloroplasts of higher plants and algae, photosynthesis converts light into biological energy, fueling the assimilation of atmospheric carbon dioxide into biologically useful molecules. Two major steps, photosynthetic electron transport and the Calvin-Benson cycle, require many gene products encoded from chloroplast as well as nuclear genomes. The expression of genes in both cellular compartments is highly dynamic and influenced by a diverse range of factors. Light is the primary environmental determinant of photosynthetic gene expression. Working through photoreceptors such as phytochrome, light regulates photosynthetic genes at transcriptional and posttranscriptional levels. Other processes that affect photosynthetic gene expression include photosynthetic activity, development, and biotic and abiotic stress. Anterograde (from nucleus to chloroplast) and retrograde (from chloroplast to nucleus) signaling

insures the highly coordinated expression of the many photosynthetic genes between these different compartments. Anterograde signaling incorporates nuclear-encoded transcriptional and posttranscriptional regulators, such as sigma factors and RNA-binding proteins, respectively. Retrograde signaling utilizes photosynthetic processes such as photosynthetic electron transport and redox signaling to influence the expression of photosynthetic genes in the nucleus. The basic C3 photosynthetic pathway serves as the default form used by most of the plant species on earth. High temperature and water stress associated with arid environments have led to the development of specialized C4 and CAM photosynthesis, which evolved as modifications of the basic default expression program. The goal of this article is to explain and summarize the many gene expression and regulatory processes that work together to support photosynthetic function in plants.

References

http://www.biologyreference.com/Ph-Po/Photosynthesis.html#ixzz4EqPp9NDG

Gene expression in the developing seed

The seed develops from the ovule and contains the embryo and endosperm, surrounded by the maternally derived seed coat. The function of the seed is to protect the embryo, to sense environmental conditions favorable to germination and to nourish the germinating seedling.

Fruits develop from organs of the flower and thus involve differentiation or redifferentiation of preexisting organs. Evolutionarily, floral organs represent modified leaves and so the fruit is also a modified leaf. Fruits serve 2 functions: to protect the seeds during development, and then to disperse the seeds following maturation.

Seed Development

All mature seeds contain an embryo and a protective covering called a seed coat (testa). In early development all angiosperm seeds also contain an endosperm, but in many seeds the endosperm is completely absorbed by the developing embryo. The embryo and endosperm are products of fertilization while the seed coat develops from the integuments of the ovule.

The seed coat contains a variety of adaptations related to protection and dispersal mechanisms. The seed coat usually forms a dry tissue. It may contain waxes for water impermeability, mucilage to make seeds sticky, compounds resistant to digestion by

animals, etc. In pomegranate, the seed coat forms the fleshy tissue that is consumed by humans. The seed coat often contains multiple layers with different characteristics.

Maternal tissues appear to have an important influence on seed development. An arabidopsis mutant called *aberrant testa shape (ats)* that lacks one of the 2 integuments also lacks several cell layers in the testa (3 layers vs. 5 normally). The seed are abnormally shaped in this mutant and seed shape shows maternal effect (ie. the genotype of the maternal parent determines the shape of the seed). Therefore, the seed coat and not the embryo determine the shape of the seed, and the embryo just grows to fill in the shape determined by the testa.

Another maternal gene called *FBP7* is specifically expressed in the ovule and seed coat and is required for normal ovule development. Downregulation of this gene in transgenic plants resulted in degeneration of the endosperm that was dependent on maternal genotype. This demonstrates the interaction between maternal tissues and those produced by fertilization.

The importance of global gene regulation

Several genes have been identified that negatively regulate seed development until fertilization has occurred. A mutant screen on a sterile line identified 3 genes that regulate seed development. Seeds develop on these mutants in the absence of fertilization. They are called *fis* for *fertilization independent seeds*. The genes appear important for control of seed development by fertilization. Several similar genes have been identified and cloned. They include:

FIE = fertilization independent endosperm, encodes a WD type POLYCOMB protein

MEDEA encodes a SET domain type POLYCOMB protein

FIS2 = fertilization independent seed2, encodes a zinc finger protein

POLYCOMB proteins are involved in chromatin structure and regulate (repress) the expression of genes in big portions of the genome. Therefore, the repression of large groups of genes is necessary to inhibit seed development until fertilization has occurred.

All three genes show parent-of-origin effects (imprinting). The maternally inherited gene is expressed and required but the paternally inherited gene is not expressed or required for seed development. (I.e. heterozygous mutants show 50% seed abortion, even when fertilized by wild type pollen [Luo, 2000 #167].

MEDEA and FIE proteins have been shown to interact by yeast 2-hybrid [Yadegari, 2000 #166].

Preparation for developmental arrest (seed/embryo maturation).

Most cell division is complete by the beginning of the maturation phase of embryo

development, but the embryo can increase in size up to 100 fold. This is by cell expansion and accompanies a massive accumulation of storage compounds. The major storage compounds are proteins, starch and lipids. These storage compounds are what give nutritional value to important crops such as cereals and beans. They are also valuable for other uses such as production of vegetable oil and starch which are used in a wide variety of ways ranging from cooking to industrial lubricants and plastics. Therefore there is a huge economic interest in seed storage compounds.

Accumulation of storage products

Storage proteins represent an important source of amino acids, nitrogen and carbon for the germinating seedling. Storage protein mRNAs represent up to 20% of the total mRNA found in a maturation phase embryo. They are synthesized on the RER and accumulate in the vacuole or as membrane bound vesicles called protein bodies. The storage proteins are encoded by several multigene families with up to 55 different genes coding for a given storage protein. Synthesis is controlled at the transcriptional level, with a few regulatory genes each controlling particular classes of storage proteins. An example is the *opaque2* gene of maize which codes for a transcription factor.

The regulation of starch and lipid accumulation, although no less important, is less well understood. These compounds are produced by complex enzymatic pathways. Each class of compound is a mixture of molecules with different chain lengths, chain branching characteristics, levels of saturation and other chemical modifications. Thus the synthesis of these compounds is much less straight forward than storage proteins.

Acquisition of dessication tolerance

At the end of embryonic development, most seeds dehydrate to about 5% moisture content. Such severe dehydration is lethal to most plant tissues and embryos express a developmental program that allows them to survive. Acquisition of dessication tolerance is part of the seed maturation program. Two problems faced by desiccated cells are high ionic concentrations and membrane stresses. At such low moisture levels, solutes would tend to crystallize and precipitate. Hydrophobic interactions with the aqueous solution are important for maintaining the integrity of the lipid bilayer. With no aqueous phase, the membrane becomes unstable and leaky.

A group of proteins called dehydrins are expressed in late maturation. The role for these proteins in desiccation tolerance is supported by their induction by drought stress in vegetative tissues and during desiccation of the resurrection plant, one of the few plants that can tolerate desiccation of postembryonic tissues. They are hypothesized to function in ion sequestration and in forming a protective layer for stabilizing membranes.

Coupling of morphogenetic and maturation programs

Morphogenesis and maturation appear to be controlled by independent developmental programs. Viviparous mutants fail to undergo the maturation program leading to seed dormancy but instead germinate directly. Morphogenesis in viviparous mutants is normal whereas other mutants arrested at various stages of morphogenesis undergo normal maturation as evidenced by the absence of necrosis following desiccation and the accumulation of storage proteins.

Integration of these programs involves both hormonal mechanisms and genetic programs. ABA is necessary to induce the expression of genes involved in maturation and desiccation tolerance. Viviparous mutants are either ABA deficient or insensitive. An ABA independent genetic program is also necessary to confer ABA sensitivity to the embryo and mutants in this program show ABA insensitive vivipary. The *LEC* gene, in which mutants both display seedling instead of embyro morphological characteristics and bypass embryo maturation are likely candidates for coordinating the two different programs.

Fruit Development

Contributions of different flower parts to the fruit

Most fruit develops from the ovary. In fact some schemes classify fruit derived from a single ovary as “true fruits” while “false fruits” are composed of tissues derived from flower parts other than the ovary or from more than one ovary.

In “true fruits” the outside of the fruit is called the pericarp and develops from the ovary wall. The pericarp can be dry and papery, like in maple or dandelions, woody like in nuts or fleshy as in berries (grapes and tomatoes) and stone fruits (cherries and peaches). These pericarp differences reflect adaptations to different dispersal mechanisms (eg. wind for papery pericarps, animal consumption for fleshy fruits). The fruit can contain a single seed as in corn, or many seeds like a pea pod or pumpkin. The pericarp of some fruits is further differentiated into specialized layers called exocarp, meso- and endocarp. For

example in citrus the rind is the exocarp, the white covering is the mesocarp and the juice sacs are the endocarp.

Many fruits we consider berries, such as raspberries and strawberries, are botanically not classified as berries. Raspberries are examples of aggregate fruits. Each juicy little sphere is actually an individual fruit of the same class as cherries, and what we consider as the fruit is really an aggregation of fruits.

Strawberries and apples are examples of accessory fruits, where some of the fleshy tissue is derived from flower parts other than the ovary. Strawberry fruits are actually what we consider the seeds. They are called achenes, which are dry fruits in the same category as dandelions. The fleshy part that we eat develops from the receptacle. Most of the fleshy tissue in apples develops from the hypanthium which is a region of the flower where sepals, petals and stamens are all fused to the ovary. Thus all floral organs contribute to the fleshy portion of apples.

Phases of fruit development

Fruit development can generally be considered to occur in four phases: fruit set, a period of rapid cell division, a cell expansion phase, and ripening/maturation.

Fruit set involves the decision whether to abort the ovary or proceed with fruit development. Fruit set is normally dependent on pollination. Pollen triggers fruit development indicating that positive signals are generated during pollination. In the absence of these signals, the flowers abscise. Growing pollen produces GA and application of GA can induce parthenocarpic fruit, therefore it is believed that GA is a triggering signal. Lagging slightly behind the growing pollen tube is a wave of increased auxin production by the style and then the ovary. Auxin application can also induce parthenocarpy and so it is thought that GA acts by inducing auxin production. However, most GA deficient mutants are able to produce fruit indicating that this is not the sole mechanism to induce fruit development and in an auxin insensitive tomato mutant, fruit growth is normal.

Continued fruit development usually relies on the continued presence of developing seeds. Seed abortion or removal causes fruit abortion, which can be reversed with auxin application. For example. removal of strawberry “seeds” prevents the development of the receptacle as a “fruit” but if auxin is applied following seed removal, fruit development continues. Commercial crops that produce parthenocarpic (seedless)

fruits, such as bananna, often show quantitaive or qualitative differences in GA or auxin content in the ovary when compared to nonparthenocarpic varieties.

The **phase of rapid cell division** involves all growing parts of the fruit. This is thought to be controlled by the developing seeds. The number of fertilized ovules in a fruit is correlated with both the initial cell division rate and the final size of the fruit. Also, fruits with an uneven distribution of seeds are often lopsided. There is a correlation between cytokinin levels in developing embryos and cell division in surrounding tissues but there is no direct evidence that embryo cytokinin in fact regulates fruit cell division. It is difficult to reconcile the complete development of parthenocarpic fruit with the requirement of embryos for cell division except to say that parthenocarpy represents an abnormal situation.

The cell division phase gradually shifts into the **cell expansion phase**. The rate and duration of cell division varies among fruits and also among tissues within a fruit. Tissues made up of many small cells at maturity continue dividing while tissues composed of large cells have begun expanding. In tomato the cell division phase lasts approximately 7-10 days while cell expansion lasts 6-7 weeks. Cell expansion accounts for the largest increase in fruit volume, often contributing in excess of a 100 fold size increase. Gibberellins are also associated with fruit expansion and removal of the seeds from pea pods inhibited GA biosynthesis in the pericarp. Many believe that auxins from seeds regulate cell expansion of the pericarp, but auxin application does not always compensate for seed removal, and in an auxin insensitive tomato mutant, fruit growth is normal.

Fruit ripening

Ripening represents the shift from the protective function to dispersal function of the fruit. Ripening occurs synchronously with seed and embryo maturation, as described in the lecture on embryo development. In dry fruits (cereals, nuts, dandelions) ripening consists of desiccation and is considered maturation. Ripening in fleshy fruits is designed to make the fruit appealing to animals that eat the fruit as a means for seed dispersal. Ripening involves the softening, increased juiciness and sweetness, and color changes of the fruit. Fleshy fruits are either climacteric or non-climacteric. Climacteric fruits produce a respirative burst with a concomitant burst in ethylene synthesis, as the fruits ripen. These include fruits with high degrees of flesh softening, like tomato, banana, avacado, peach etc.

Ripening has been most intensively studied in tomato. Ethylene is a major regulator of the ripening process. Inhibitioin of ethylene with inhibitors, transgenic approaches or

mutants blocks ripening. Exogenous ethylene accelerates ripening. There are also developmental factors involved because fruit does not attain competence to respond to ethylene until near the end of the cell expansion phase (the mature green stage). Several genes associated with ripening are ethylene inducible. This occurs transcriptionally in most genes but at least one is known where mRNA accumulation is regulated post-transcriptionally. None of these genes are induced until competence for ethylene response is attained.

The tomato *never-ripe* mutation blocks fruit ripening and is insensitive to ethylene. The mutated gene is similar to the ethylene receptor isolated from arabidopsis, suggesting that *never-ripe* is an ethylene receptor mutant. *NR* mRNA is not expressed until the mature green stage, suggesting that lack of this ethylene receptor might be related to the lack of competence to respond to ethylene at earlier stages.

Ethylene production is autocatalytic. That is, exposure to ethylene stimulates the synthesis of more ethylene. This occurs because the genes for the biosynthetic enzymes (e.g. ACC SYNTHASE) are ethylene inducible. The result is a positive feedback loop. Furthermore, the *Never-ripe* gene is ethylene inducible, resulting in a positive feedback loop for ethylene sensitivity as well. Both these factors contribute to the dramatic burst of ethylene production during ripening.

Fruit softening involves a partial breakdown of cell walls. Several enzymes are known to be involved in this process. Polygalacturonase hydrolyzes bonds in pectins. The gene for this enzyme is ethylene inducible.

Changes in fruit color involve changes in the expression of pigment biosynthetic genes. The major pigment in tomato is a carotenoid. The first committed step in carotenoid biosynthesis is catalyzed by phytoene synthase, and the gene for this enzyme is induced by ethylene.

Germination

Seeds have mechanisms to ensure germination occurs only under favorable environmental conditions for seedling growth. The primary factors are water availability and season. All seeds must imbibe water to germinate and for some this is the only requirement. Some also contain growth inhibitors that must be leached out of the seed. Some have impervious seed coats that must be fractured by freezing or passage through the digestive tract of an animal. Yet others have light or photoperiod requirements. All these mechanisms ensure the seeds germinate in the correct season and when moisture is available.

Arabidopsis seeds have certain requirements for germination, including a period of dormancy (which can be substituted for by cold treatment) and light (a phytochrome response). Mutations in a gene called *DAG1* (*Dof Affecting Germination1*) cause seeds that germinate in the dark without a dormancy period. Dof proteins are zinc finger transcription factors. The gene is expressed in the maternal tissues and all seeds of a mutant show this phenotype even if they result in pollination by a wild type (i.e. the embryo is wild type). Therefore, the maternal tissues during seed development control the dormancy behavior of the seed after being shed from the plant.

Upon imbibition, active metabolism resumes. Imbibed seeds contain high levels of GA. It is produced by the germinating embryo and stimulates the synthesis of hydrolytic enzymes by inducing the transcription of their genes. These enzymes appear after radicle elongation and are therefore postgerminative. The hydrolytic enzymes include proteases, amylases and lipases that break down storage compounds making building blocks available to the growing seedling. One enzyme of particular importance is a- amylase which cleaves starch into glucose and maltose molecules. This reaction is of economic importance to the malting industry and so the regulation of a-amylase gene expression has been carefully studied. It is transcriptionally induced by GA. Plants also contain a unique metabolic pathway called the glyoxylate cycle. This enables plants to convert fatty acids of the stored lipids into carbohydrates, specifically glucose and sucrose. In contrast, animals are unable to convert fatty acids to glucose.

GA and ABA act antagonistically to regulate the germination vs. maturation programs. ABA promotes maturation while GA promotes germination. As mentioned, ABA is necessary for seed maturation because ABA deficient mutants are viviparous and desiccation intolerant. Therefore, without ABA, seeds directly enter the germination program. Exogenous ABA can inhibit germination following dormancy. Conversely, promotes germination. GA is required for germination because GA deficient mutants are unable to germinate. Exogenous GA application to developing seeds can block maturation and induce vivipary. The VP1/ABI3 protein is a central regulator in these functions. This protein is a transcription factor that promotes the expression of maturation genes and inhibits the expression of germination genes. Mutants in this gene are ABA insensitive.