PLANT PHYSIOLOGY

Vince Ördög

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Table 1.

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Chapter 1. Preface

Plant physiology is one chapter from the classical handbook of Strasburger (2008). According to him, plant physiology is the science which is connected to the material and energy exchange, growth and development, as well as movement of plant. Plant physiology is the science that studies plant function: what is going on in plants that accounts for their being alive (Salisbury and Ross, 1992). Another definition of plant physiology by Taiz and Zeiger (2010) is the study of plant function, encompassing the dynamic processes of growth, metabolism and reproduction in living plants. Nowadays these latter two handbooks are widely used in the European higher educational level.

Plant physiology is overlapped with its related branch of knowledge: biochemistry, biophysics, and molecular biology. The basic knowledge of plant physiology, that is necessary for experts in agriculture, is presented in our lecture notes based on the content of the above mentioned three handbooks, complemented with Hopkins and Hüner's (2009) manual. Uptake and transport of water and minerals are explained in general. The nutrient supply of plant is presented in details (essential elements, solute transport, nutritional deficiencies). Most common processes of plant biochemistry and metabolism, such as photosynthesis, are highlighted. Plant growth and development is introduced with the characterization and commercial use of plant growth regulators (PGRS, plant hormones). The basic concepts of plant stress is complemented with the presentation of physiological mechanisms against different environmental stresses.

Chapter 2. Water and nutrients in plant

1. Water balance of plant

Water in plant life

Water plays a crucial role in the life of plant. It is the most abundant constituents of most organisms. Water typically accounts for more than 70 percent by weight of non-woody plant parts. The water content of plants is in a continual state of flux. The constant flow of water through plants is a matter of considerable significance to their growth and survival. The uptake of water by cells generates a pressure known as **turgor**. Photosynthesis requires that plants draw carbon dioxide from the atmosphere, and at the same time exposes them to water loss. To prevent leaf desiccation, water must be absorbed by the roots, and transported through the plant body. Balancing the uptake, transport, and loss of water represents an important challenge for land plants. The thermal properties of water contribute to temperature regulation, helping to ensure that plants do not cool down or heat up too rapidly. Water has excellent solvent properties. Many of the biochemical reactions occur in water and water is itself either a reactant or a product in a large number of those reactions.

The practice of crop irrigation reflects the fact that water is a key resource limiting agricultural productivity. Water availability likewise limits the productivity of natural ecosystems (**Figure 1.1**). Plants use water in huge amounts, but only small part of that remains in the plant to supply growth. About 97% of water taken up by plants is lost to the atmosphere, 2% is used for volume increase or cell expansion, and 1% for metabolic processes, predominantly photosynthesis. Water loss to the atmosphere appears to be an inevitable consequence of carrying out photosynthesis. The uptake of CO2 is coupled to the loss of water (**Figure 1.2**). Because the driving gradient for water loss from leaves is much larger than that for CO2 uptake, as many as 400 water molecules are lost for every CO2 molecule gained.

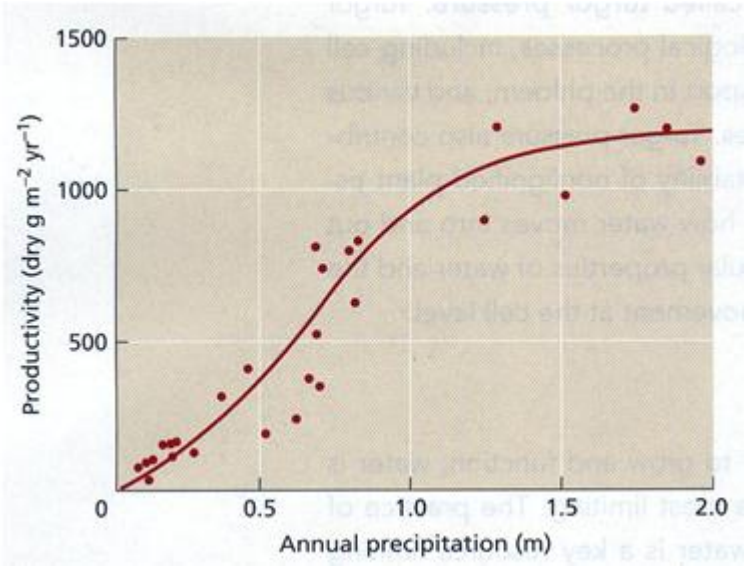


Figure 1.1 Productivity of various ecosystems as a function of annual precipitation (*source: Taiz L., Zeiger E., 2010*)

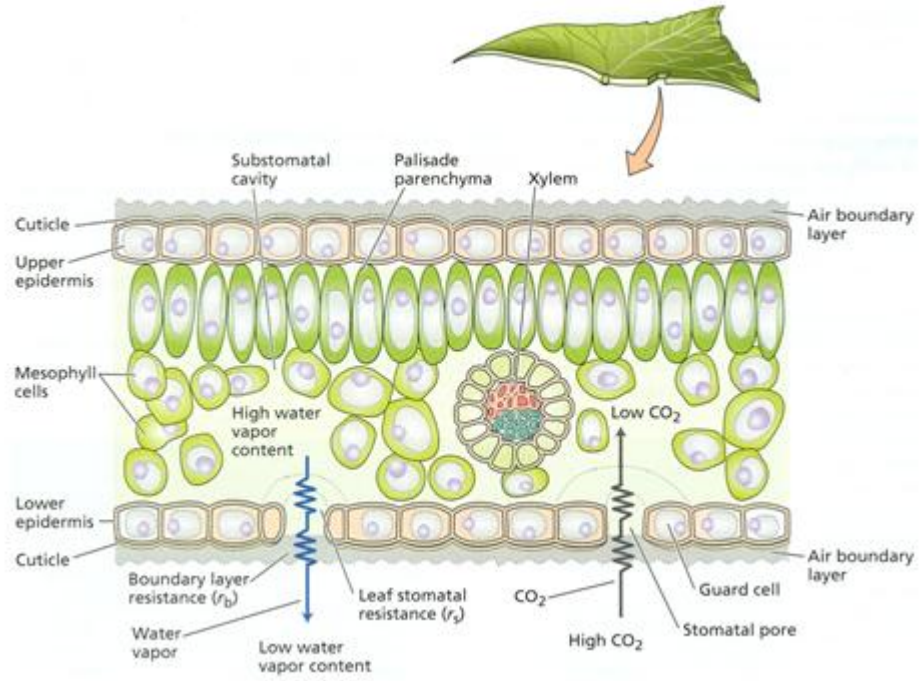


Figure 1.2 Water pathway through the leaf (*source: Taiz L., Zeiger E., 2010*)

1.1. Water potential

The structure and properties of water

Water consists of an oxygen atom covalently bonded to two hydrogen atoms (**Figure 1.3**). The oxygen atom carries a partial negative charge, and a corresponding partial positive charge is shared between the two hydrogen atoms. This asymmetric electron distribution makes water a **polar molecule**. However, the partial charges are equal, and the water remains a neutral molecule. There is a strong electrical attraction between adjacent water molecules or between water and other polar molecules, which is called hydrogen bonding. The **hydrogen bonding** ability of water and its polar structure make it a particularly good solvent for ionic substances and for molecules such as sugars and proteins. The hydration shells that form around biologically important macromolecules are often referred to as **bound water**. Bound water prevents protein molecules from approaching close enough to form aggregates large enough to precipitate.

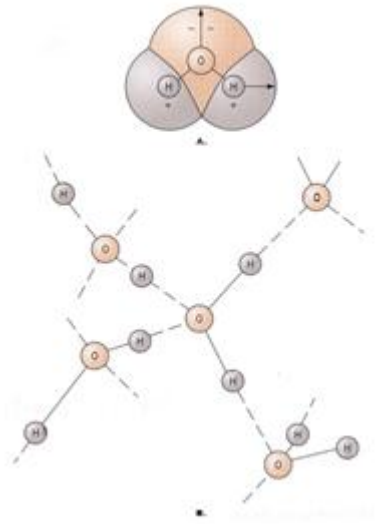


Figure 1.3 A) Structure of a water molecule B) Hydrogen bonds among water molecules (*source: Hopkins W.G., Hüner N.P.A., 2009*)

The extensive hydrogen bonding between water molecules results in water having both a high **specific heat capacity** and a high **latent heat of vaporization**. Because of its highly ordered structure, liquid water also has a high **thermal conductivity**. This means that it rapidly conducts heat away from the point of application. The combination of high specific heat and thermal conductivity enables water to absorb and redistribute large amounts of heat energy without correspondingly large increases in temperature. The heat of biochemical reactions may be quickly dissipated throughout the cell. Compared with other liquids, water requires a relatively large heat input to raise its temperature. This is important for plants, because it helps buffer temperature fluctuations. The latent heat of vaporization decreases as temperature increases, reaching a minimum at the boiling point (100°C). For water at 25°C, the heat of vaporization is 44kJ mol-1 – the highest value known for any liquid.

The excellent solvent properties of water are due to the highly polar character of the water molecule. The polarity of molecules can be measured by a quantity known as the **dielectric constant**. Water has one of the highest dielectric constant, which is as high as 78.4. The dielectric constant of benzene and hexane is 2.3 and 1.9, respectively. Water is thus an excellent solvent for charged ions or molecules, which dissolve very poorly in non-polar organic liquids.

The extensive hydrogen bonding in water gives a new property known as **cohesion**, the mutual attraction between molecules. A related property, called **adhesion**, is the attraction of water to a solid phase, such as cell wall. The water molecules are highly cohesive. One consequence of cohesion is that water has exceptionally high **surface tension**, which is the energy required to increase the surface area of a gas-liquid interface. Surface tension and adhesion at the evaporative surfaces in leaves generate the physical forces that pull water through the plant's vascular system. Cohesion, adhesion and surface tension give rise to a phenomenon known as **capillarity**. These combined properties of water help to explain why water rises in capillary tubes and are exceptionally important in maintaining the continuity of water columns in plants.

Hydrogen bonding gives water a high tensile strength, defined as the maximum force per unit area that a continuous column of water can withstand before breaking. Water can resist pressures more negative than -20 MPa, where the negative sign indicates tension, as opposed to compression. Pressure is measured in units called pascals (Pa), or more conveniently, megapascals (MPa). One MPa equals approximately 9.9 atmospheres.

Water movement by diffusion, osmosis and bulk flow

Movement of substances from one region to another is commonly referred to as translocation. Mechanisms for translocation may be classified as either active or passive. It is sometimes difficult to distinguish between active and passive transport, but the translocation of water is clearly a passive process. Passive movement of most substances can be accounted for by **bulk flow** or **diffusion**. The diffusion of water across a selectively permeable barrier is known as **osmosis**, which must also be taken into account.

Bulk flow accounts for some water movement in plants through the xylem tissues of plants. Movement of materials by bulk flow (or mass flow) is pressure driven. Bulk flow occurs when an external force, such as gravity or pressure, is applied. As a result, all of the molecules of the substance move in mass. Bulk flow is pressure-driven, diffusion is driven principally by concentration differences.

The molecules in a solution are not static, they are in continuous motion. Diffusion results in the net movement of molecules from regions of high concentration to regions of low concentration. This tendency for a system to evolve toward and even distribution of molecules can be understood as a consequence of the second law of thermodynamics, which tells us that spontaneous processes evolve in the direction of increasing entropy or disorder. Diffusion represents the natural tendency of systems to move toward the lowest possible energy state. Fick's first law describes the process of diffusion, which is most effective over short distances. Diffusion in solutions can be effective within cellular dimensions but is far too slow to be effective over long distances. The average time required for a glucose molecule to diffuse across a cell with a diameter of 50 μm is 2.5 s. However, the average time needed for the same glucose molecule to diffuse a distance of 1 m in water is approximately 32 years.

The net movement of water across a selectively permeable barrier is referred to as osmosis. Membranes of plant cells are selectively permeable. The diffusion of water directly across the lipid bilayer is facilitated by **aquaporins**, which are integral membrane proteins that form water-selective channels across membrane. In osmosis the maximization of entropy is realized by the volume of solvent diffusing through the membrane to dilute the solute. Osmosis can be easily demonstrated using a device known as an osmometer, constructed by closing off the open end of a thistle tube with a selectively permeable membrane (**Figure 1.4**). If the tube is

filled with a sugar solution and inverted in a volume of pure water, the volume of solution in the tube will increase over time. The increase in the volume of the solution will continue until the hydrostatic pressure developed in the tube is sufficient to balance the force driving the water into the solution.

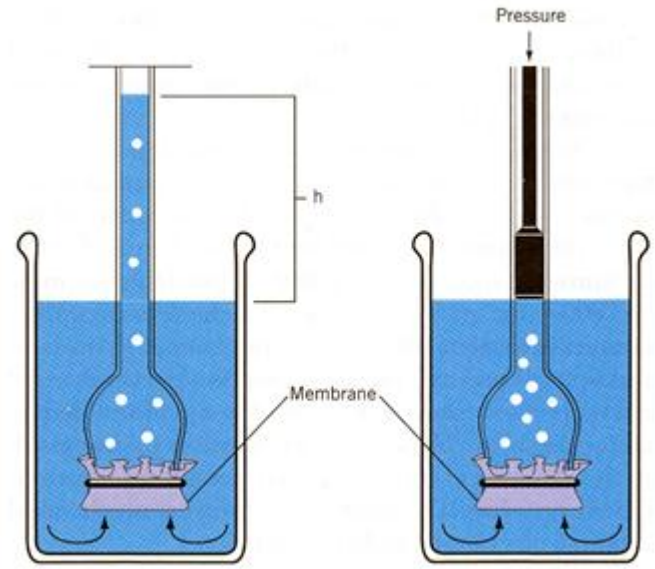


Figure 1.4 A demonstration of hydrostatic pressure (*source: Hopkins W.G., Hüner N.P.A., 2009*)

The concept of water potential

All living things, including plants, require a continuous input of free energy to maintain and repair their highly organised structures, as well as to grow and reproduce. Chemical potential is a quantitative expression of the free energy associated with a substance. The chemical potential of the water represents the free energy associated with water. Water flows without energy input from regions of higher chemical potential to ones of lower chemical potential. The concept of water potential was introduced in 1960 by R.O. Slatyer and S.A. Taylor, as a measure of the free energy of water per unit volume (J m-3). These units are equivalent to pressure units such as the pascal, which is the common measurement unit for water potential.

The major factors influencing the water potential in plants are *concentration, pressure and gravity*. Water potential is symbolized by Ψw (the Greek letter psi), and the water potential of solutions may be dissected into individual components, usually written as the following sum:

$\Psi w = \Psi s + \Psi p + \Psi g$

The terms Ψs and Ψp and Ψg denote the effects of solutes, pressure, and gravity, respectively, on the free energy of water. The reference state most often used to define water potential is pure water at ambient temperature and standard atmospheric pressure.

The term Ψs, called the **solute potential** or the **osmotic potential**, represents the effect of dissolved solutes on water potential. Solutes reduce the free energy of water by diluting the water. It's value is negative or maximum zero. The minus sign indicates that dissolved solutes reduce the water potential of a solution relative to the reference state of pure water. Osmosis can be easily demonstrated using a device known as osmometer. The increase in the volume of the solution will continue until the hydrostatic pressure developed in the tube of the osmometer is sufficient to balance the force driving the water into the solution. This force, measured in units of pressure, is known as osmotic pressure. It is convention to define osmotic potential as the negative of the osmotic pressure, since they are equal but opposite forces.

The term Ψp is the **hydrostatic pressure** of the solution. Positive pressures raise the water potential; negative pressures reduce it. The positive hydrostatic pressure within cells is the turgor pressure. Negative hydrostatic pressure (**tension**) develops in the xylem and in the walls between cells. Gravity causes water to move downward unless the force of gravity is opposed by an equal and opposite force. The term Ψg depends on the height (h) of the water above the reference state water. The gravitational component (Ψg) of the water potential

is generally omitted in considerations of water transport in the cell level. Thus in these cases the equation can be simplified as follows:

$\Psi w = \Psi s + \Psi p$

Water potentials can be measured by different methods, among others by the Sholander's pressure chamber (**Figure 1.5**). In this technique, the organ to be measured is excised from the plant and is partly sealed in a pressure chamber. Before excision, the water column in the xylem is under tension. When the water column is broken by excision of the organ (i.e., its tension is relieved allowing its ΨP to rise to zero), water is pulled rapidly from the xylem into the surrounding living cells by osmosis. The cut surface consequently appears dull and dry. To make a measurement, the investigator pressurizes the chamber with compressed gas until the distribution of water between the living cells and the xylem conduits is returned to its initial, pre-excision, state. This can be detected visually by observing when the water returns to the open ends of the xylem conduits that can be seen in the cut surface. The pressure needed to bring the water back to its initial distribution is called the balance pressure and is readily detected by the change in the appearance of the cut surface, which becomes wet and shiny when this pressure is attained. Pressure chamber measurements provide a quick and accurate way of measuring leaf water potential. Because the pressure chamber method does not require delicate instrumentation or temperature control, it has been used extensively under field conditions.

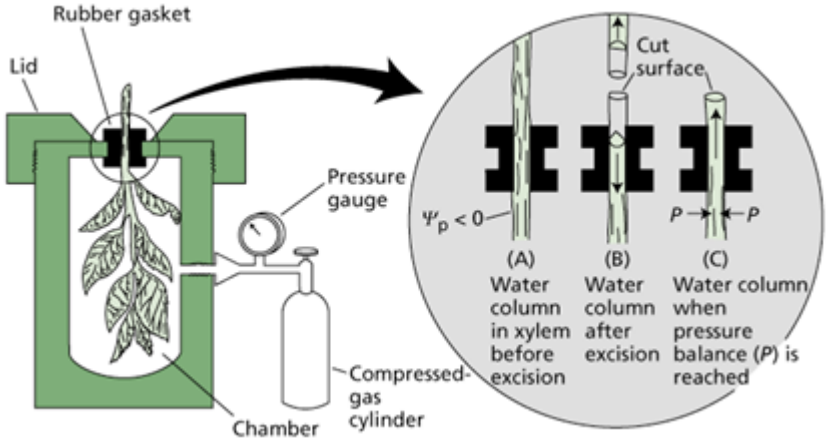


Figure 1.5 The pressure chamber method for measuring plant water potential (*source: Taiz L., Zeiger E., 2010*)

Cell growth, photosynthesis, and crop productivity are all strongly influenced by water potential and its components. Plant scientists have thus expended considerable efforts in devising accurate and reliable methods for evaluating the water status of plants. Plant cells typically have water potentials ≤0 MPa. A negative value indicates that the free energy of water within the cell is less than that of pure water. In leaves of well-watered plants, Ψw ranges from -0.2 to about -1.0 MPa in herbaceous plants and to 2.5 MPa in trees and shrubs. Within cells of well-watered garden plants (examples include lettuce, cucumber seedlings, and bean leaves) Ψs may be as high as 0.5 MPa (low cell solute concentration), although values of -0.8 to -1.2 MPa are more typical. The Ψs of the apoplast is typically -0.1 to 0 MPa. In general, water potentials in the xylem and cell walls are dominated by Ψp, which is typically less than zero. Values for Ψp within cells of well-watered plants may range from 0.1 to as much as 3 MPa. The plant **wilts** when the turgor pressure inside the cells of such tissues falls toward zero.

1.2. Absorption by roots

Water in the soil

The water content and the rate of water movement in soils depend to a large extent on soil type and soil structure. Like the water potential of the plant cells, the water potential of soils may be dissected into three components: the osmotic potential, the hydrostatic pressure and the gravitational potential. The osmotic potential (Ψs) of soil water is generally negligible. The second component of soil water potential is hydrostatic pressure (Ψp). For wet soils, Ψp is very close to zero. As soil dries out Ψp decreases and can become quite negative. As the water content of the soil decreases, the water recedes into the interstices between soil particles, forming air-water surfaces whose curvature represents the balance between the tendency to minimize the surface area of the air-water interface and the attraction of the water for the soil particles. Water under a curved surface develops a negative pressure (like in leaf mesophyll). As soil dries out, water is first removed from the largest

spaces between soil particles. The value of Ψp may easily reach -1 to -2 MPa as the air-water interface recedes into the smaller spaces between clay particles. The third component is gravitational potential (Ψg). Gravity plays an important role in drainage.

Water absorption by roots

Intimate contact between the surface of root and the soil is essential for effective water absorption. **Root hairs** are filamentous outgrowths of root epidermal cells that greatly increase the surface area of the root, thus providing greater capacity for absorption of ions and water from the soil (**Figure 1.6**). Water enters the root most readily near the root tip. The intimate contact between the soil and the root surface is easily ruptured when the soil is disturbed. It is for this reason that newly transplanted seedlings and plants need to be protected from water loss for the first few days after transplantation.

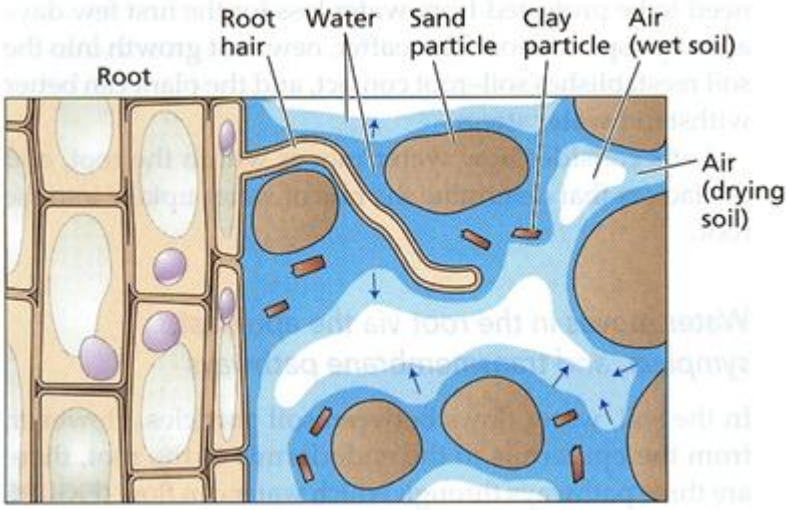


Figure 1.6 Root hairs intimate contact with soil particles and greatly amplify the surface area used for water absorption by the plant (*source: Taiz L., Zeiger E., 2010*)

From the epidermis to the endodermis of the root, there are three pathways through which water can flow: the apoplast, the symplast and the transmembrane pathway (**Figure 1.7**).

1. The apoplast is the continuous system of cell walls and intercellular air spaces. In this pathway water moves without crossing any membranes as it travels across the root cortex.

2. The symplast consists of the entire network of cell cytoplasm interconnected by plasmodesmata. In this pathway, water travels across the root cortex via the plasmodesmata.

3. The transmembrane pathway is the route by which water enters a cell on one side, exits the cell on the other side, enters the next in the series, and so on. In this pathway, water crosses the plasma membrane of each cell in its path twice.

Though there are three pathways, water moves not according to a single chosen path, but wherever the gradients and resistances direct it. At the endodermis the Casparian strip breaks the continuity of the apoplast pathway, forcing water and solutes to pass through the plasma membrane in order to cross the endodermis. The requirement that water move symplastically across the endodermis helps explain why the permeability of roots to water depends strongly on the presence of aquaporins.

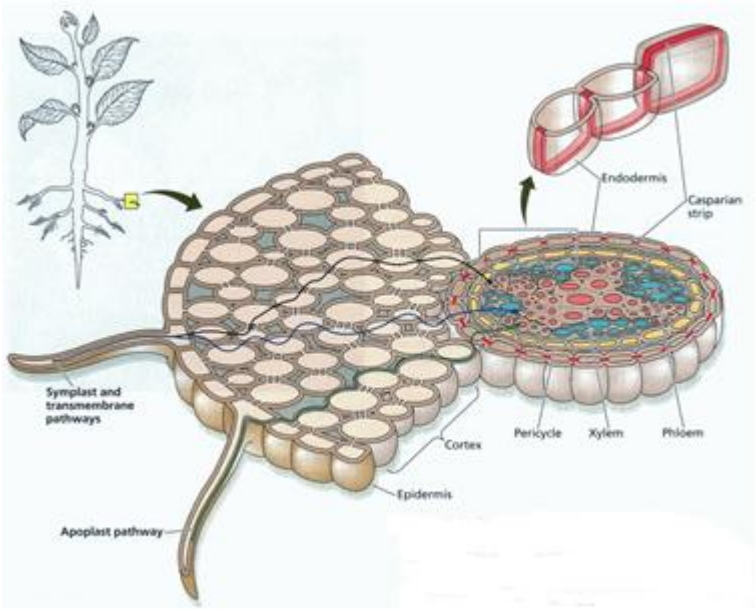


Figure 1.7 Pathways (symplast, transmembrane and apoplast) for water uptake by the root (*source: Taiz L., Zeiger E., 2010*)

Water uptake decreases when roots are subjected to low temperature or anaerobic conditions. Decreased rate of respiration, in response to low temperature or anaerobic conditions, can lead to increases in intracellular pH. This increase in cytoplasmic pH alters the conductance of aquaporins in root cells, resulting in roots that are markedly less permeable to water.

Plants sometimes exhibit a phenomenon referred to as **root pressure**. If the stem of a young seedling is cut off just above the soil, the stump will often exude sap from the cut xylem for many hours. If a manometer is sealed over the stump, positive pressures as high as 0.05 to 0.2 MPa can be measured. Plants that develop root pressure frequently produce liquid droplets on the edges of their leaves, a phenomenon known as **guttation**. Guttation is most noticeable when transpiration is suppressed and the relative humidity is high, such as at night.

1.3. Transport through the xylem

Vascular tissues include the xylem and phloem, which conduct water and nutrients between the various organs. In leaves, the larger veins subdivide into smaller veins such that no photosynthetic leaf cell is more than a few cells removed from a small vein ending. Xylem tissue is responsible for the transport of water and dissolved minerals from the root to the stem to aerial organs. Phloem, on the other hand, is responsible primarily for the translocation of organic materials from sites of synthesis to storage sites or sites of metabolic demand.

Transpiration speeds up the movement of xylem sap, but it seems unlikely that this is an essential requirement. Transpiration involves the evaporation of water, it can assume a significant role in the cooling of leaves. However, the main evolutionary function of stomata is to ensure an adequate supply of carbon dioxide for photosynthesis

The xylem consists of two types of tracheary elements

There are two main types of **tracheary elements** in the xylem: tracheids and vessel elements. Vessel elements are found in angiosperms. Tracheids are present in both angiosperms and gymnosperms. Both tracheids and vessel elements dead cells with thick, lignified cell walls, which form hollow tubes through which water can flow with relatively little resistance. **Tracheids** are elongated, spindle-shaped cells that are arranged in overlapping vertical files. **Vessel elements** tend to be shorter and wider than tracheids and have perforated end walls that form a perforation plate at each end of the cell.

Water moves through the xylem by pressure-driven bulk flow

Pressure-driven bulk flow of water is responsible for long-distance transport of water in the xylem. It is independent of solute concentration gradient, as long as viscosity changes are negligible. It is extremely

sensitive to the radius of the tube. If the radius is doubled, the volume of flow rate increases by a factor of 16 (24). Vessel elements up to 500 μm in diameter are, nearly an order of magnitude greater than the largest tracheids.

The cohesion-tension theory explains water transport in the xylem

In theory, the pressure gradients needed to move water through the xylem could result from the generation of positive pressures at the base of the plant or negative pressures at the top of the plant. However, root pressure is typically less than 0.1 MPa and disappears when the transpiration rate is high or when soils are dry, so it is clearly inadequate to move water up a tall tree. Instead, the water at the top of a tree develops a large tension (negative hydrostatic pressure), and this tension pulls water through the xylem (**Figure 1.8**). This mechanism, first proposed toward the end of the nineteenth century, is called the cohesion-tension theory of sap ascent because it requires the cohesive properties of water to sustain large tensions in the xylem water column. The theory is generally credited to H.H. Dixon, who gave the first detailed account of it in 1914.

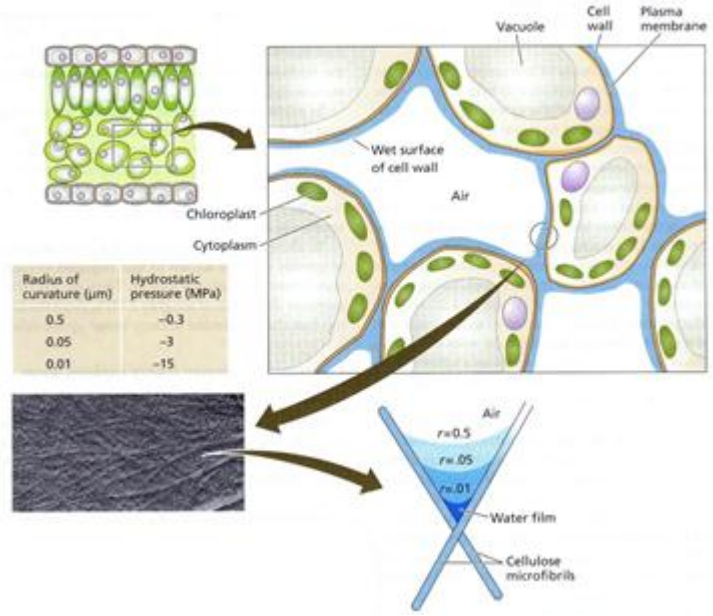


Figure 1.8 The driving force for water movement through plants originates in leaves (*source: Taiz L., Zeiger E., 2010*)

The negative pressure that causes water to move up through the xylem develops at the surface of the cell walls in the leaf. As water evaporates from mesophyll cells within the leaf, the surface of the remaining water is drawn into the interstices of the cell wall, where it forms curved air interfaces. Because of the high surface tension of water, the curvature of these interfaces induces a tension, or negative pressure, in water. The cohesion-tension theory explains how the substantial movement of water through plants occur without the direct expenditure of metabolic energy.

1.4. Transpiration

Water movement is determined by differences in water potential. It can be assumed that the driving force for transpiration is the difference in water potential between the substomatal air space and the external atmosphere. However, because the problem is now concerned with the diffusion of water vapour rather than liquid water, it will be more convenient to think in terms of vapour systems. We can say that when a gas phase has reached equilibrium and is saturated with water vapour, the system will have achieved its **saturation vapour pressure**. The vapour pressure over a solution at atmospheric pressure is influenced by solute concentration and mainly by temperature. In principle we can assume that the substomatal air space of leaf is normally saturated or very nearly saturated with water vapour. On the other hand, the atmosphere that surrounds the leaf is usually unsaturated and may often have a very low water content. This difference in water vapour pressure between the internal air spaces of the leaf and the surrounding air is the driving force of transpiration.

On its way from the leaf to the atmosphere, water is pulled from the xylem into the cell walls of the mesophyll, where it evaporates into the air spaces of the leaf. The water vapor than exits the leaf through the stomatal pore.

The movement of liquid water through the living tissues of the leaf is controlled by gradients in water potential. However, transport in the vapor phase is by diffusion, so the final part of the transpiration stream is controlled by the concentration gradient of water vapor. Almost all of the water lost from leaves is lost by diffusion of water vapour through the tiny stomatal pores. The stomatal transpiration accounts for 90 to 95% of water loss from leaves. The remaining 5 to 10% is accounted for by cuticular transpiration. In most herbaceous species, stomata are present in both the upper and lower surfaces of the leaf, usually more abundant on the lower surface. In many tree species, stomata are located only on the lower surface of the leaf.

The driving force for transpiration is the difference in water vapour concentration

Transpiration from the leaf depends on two major factors: (1) the **difference in water vapor concentration** between the leaf air spaces and the external bulk air and (2) the **diffusional resistance** of this pathway. Air space volume is about 10% in corn leaves, 30% in barley, and 40% in tobacco leaves. In contrast to the volume of the air space, the internal surface area from which water evaporates may be from 7 to 30 times the external leaf area. The air space in the leaf is close to water potential equilibrium with the cell wall surfaces from which liquid water is evaporating. The concentration of water vapor changes at various points along the transpiration pathway from the cell wall surface to the bulk air outside the leaf.

The second important factor governing water loss from the leaf is the diffusional resistance of the transpiration pathway, which consists of two varying components:

1. The resistance associated with diffusion through the stomatal pore, the **leaf stomatal resistance**.

2. The resistance due to the layer of unstirred air next to the leaf surface through which water vapor must diffuse to reach the turbulent air of the atmosphere. This second resistance is called the leaf boundary layer resistance.

Some species are able to change the orientation of their leaves and thereby influence their transpiration rates. Many grass leaves roll up as they experience water deficits, in this way increasing their boundary layer resistance.

Stomatal control couples leaf transpiration to leaf photosynthesis

Because the cuticle covering the leaf is nearly impermeable to water, most leaf transpiration results from the diffusion of water vapor through the stomatal pore. The microscopic stomatal pores provide a low-resistance pathway for diffusional movement of gases across the epidermis and cuticle. Changes in stomatal resistance are important for the regulation of water loss by the plant and for controlling the rate of carbon dioxide uptake necessary for sustained CO2 fixation during photosynthesis. At night, when there is no photosynthesis and thus no demand for CO2 inside the leaf, stomatal apertures are kept small or closed, preventing unnecessary loss of water. Leaf can regulate its stomatal resistance by opening and closing of the stomatal pore. This biological control is exerted by a pair of specialized epidermal cells, the **guard cells**, which surround the stomatal pore.

The cell walls of guard cells have specialized features

Guard cells are found in leaves of all vascular plants. In grasses, guard cells have a characteristic dumpbell shape, with bulbous ends (**Figure 1.9**). These guard cells are always flanked by a pair of differentiated epidermal cells called **subsidiary cells**, which help the guard cells control the stomatal pores. In dicots and nongrass monocots, guard cells have an elliptical contour (often called “kidney-shaped”) with the pore at their center. Subsidiary cells are often absent, the guard cells are surrounded by ordinary epidermal cells. A distinctive feature of guard cells is the specialized structure of their walls. The alignment of cellulose microfibrils, which reinforce all plant cell walls and are an important determinant of cell shape, play an essential role in the opening and closing of the stomatal pore.

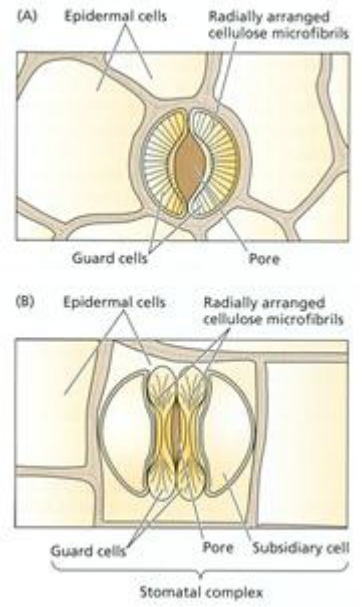


Figure 1.9 The radial alignment of the cellulose microfibrils in guard cells and epidermal cells of (A) a kidney-shaped stoma and (B) a grasslike stoma (*source: Taiz L., Zeiger E., 2010*)

An increase in guard cell turgor pressure opens the stomata

Guard cells function as multisensory hydraulic valves. Environmental factors such as light intensity and quality, temperature, leaf water status, and intracellular CO2 concentrations are sensed by guard cells, and these signals are integrated into well-defined stomatal responses. The early aspects of this process are ion uptake and other metabolic changes in the guard cells. The decrease of osmotic potential (Ψs) resulting from ion uptake and from biosynthesis of organic molecules in the guard cells. Water relations in guard cells follow the same rules as in other cells. As Ψs decreases, the water potential decreases, and water consequently moves into the guard cells. As water enters the cell, turgor pressure increases. Because of the elastic properties of their walls, guard cells can reversible increase their volume by 40 to 100%, depending on the species. Such changes in cell volume lead to opening or closing of the stomatal pore. Subsidiary cells appear to play an important role in allowing stomata to open quickly and to achieve large apertures.

The transpiration ratio measures the relationship between water loss and carbon gain

The effectiveness of plants in moderating water loss while allowing sufficient CO2 uptake for photosynthesis can be assessed by a parameter called the **transpiration ratio**. This value is defined as the amount of water transpired by the plant divided by the amount of carbon dioxide assimilated by photosynthesis. For plants in which the first stable product of carbon fixation is a 3-carbon compound (C3 plants), as many as 400 molecules of water are lost every molecule of CO2 fixed by photosynthesis, giving a transpiration ratio of 400. Plants in which a 4-carbon compound is the first stable product of photosynthesis (C4 plants), generally transpire less water per molecule of CO2 fixed than C3 plants do. A typical transpiration ratio for C4 plants is about 150. Plants with crassulacean acid metabolism (CAM) photosynthesis the transpiration ratio is low, values of about 50 are not unusual.

1.5. Plant water status

The water status of plant cells is constantly changing as the cells adjust to fluctuations in the water content of the environment or to changes in metabolic state. The plant water status is dependent on: the soil moisture content, the capacity for water absorption by roots, and the hydraulic conductivity of root and shoot tissues. Water potential is often used as a measure of the water status of a plant. Plants are seldom fully hydrated. During periods of drought, they suffer from water deficits that lead to inhibition of plant growth and photosynthesis. Several physiological changes occur as plants experience increasingly drier conditions (**Figure 1.10**). Cell expansion is most affected by water deficit. In many plants reductions in water supply inhibit shoot growth and leaf expansion but stimulate root elongation. Drought does impose some absolute limitations on physiological processes, although the actual water potentials at which such limitations occur vary with species.

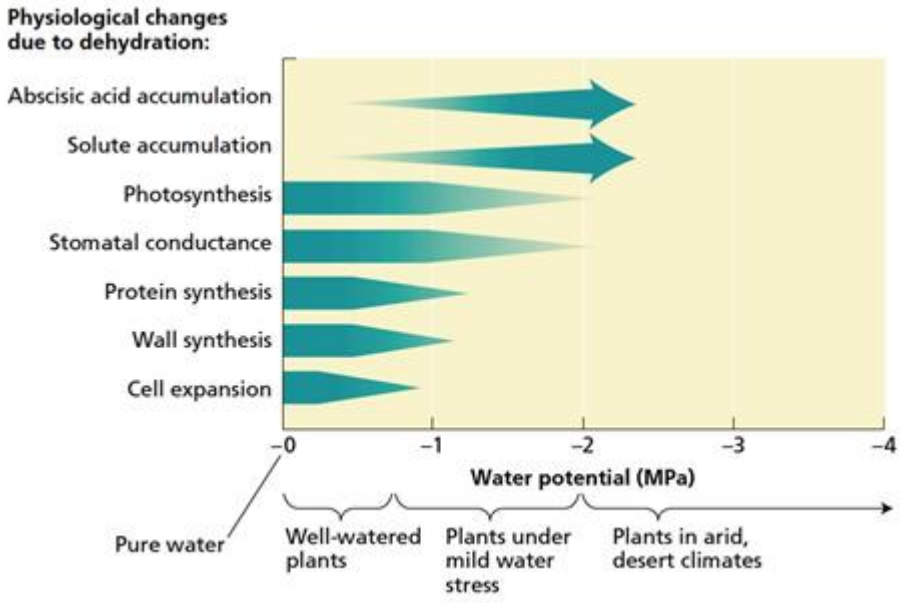


Figure 1.10 Sensitivity of various physiological processes to changes in water potential under various growing conditions (*source: Taiz L., Zeiger E., 2010*)

The plant may *spend energy* to accumulate solutes to maintain turgor pressure, invest in the growth of non-photosynthetic organs such as roots to increase water uptake capacity, or build xylem conduits capable of withstanding large negative pressures. Thus, physiological responses to water availability reflect a trade-off between the benefits accrued by being able to carry out physiological processes (e.g., growth) over a wider range of environmental conditions and the costs associated with such capability.

Water stress typically leads to an *accumulation of solutes* in the cytoplasm and vacuole of plant cells, thus allowing the cells to maintain turgor pressure despite low water potential. Some physiological processes appear to be influenced directly by turgor pressure. However, the existence of stretch-activated signalling molecules in the plasma membrane suggests that plant cells may sense changes in their water status via changes in volume, rather than by responding directly to turgor pressure.

1.6. Influence of extreme water supply

Plant growth can be limited both by water deficit and by excess water. *Drought* is the meteorological term for a period of insufficient precipitation that results in plant water deficit. *Excess water* occurs as the result of flooding or soil compaction. The deleterious effects of excess water are a consequence of the displacement of oxygen from the soil.

When soil is water-saturated, the water potential (Ψw) of the soil solution may approach zero, but drying can reduce the soil Ψw to below -1.5 MPa, the point at which *permanent wilting* can occur. The relative humidity of the air determines the vapour pressure gradient between the leaf stomatal cavity and the atmosphere, and this vapour pressure gradient is the driving force for transpirational water loss.

When a soil dries, its hydraulic conductivity decreases very sharply, particularly near the permanent wilting point (that is, the soil water content at which plant cannot regain turgor upon rehydration). Redistribution of water within the roots often occurs at night, when evaporative demand from leaves is low. Water-deficient plants tend to become rehydrated at night, allowing leaf growth during the day. But at the permanent wilting point, water delivery to the roots is too slow to allow the overnight rehydration of plants that have wilted during the day. Thus, decreasing soil water conductivity hinders rehydration after wilting.

Water deficit is stressful, but too much water can also have several potentially negative consequences for a plant. Flooding and soil compaction result in poor drainage, leading to reduced O2 availability to cells. Flooding fills soil pores with water, reducing O2 availability. Dissolved oxygen diffuses so slowly in stagnant water that only a few cm of soil near the surface remain oxygenated. At low temperatures the consequences are relatively harmless. However, when temperatures are higher (greater than 20°C), O2 consumption by plant roots, soil fauna, and microorganisms can totally deplete O2 from the soil in as little as 24 hours. Flooding sensitive plants

are severely damaged by 24 hours of anoxia (lack of oxygen). The yield of flooding-sensitive garden-pea (*Pisum sativum*) may decrease by fifty percent. Corn is affected by flooding in a milder way, and is more resistant to flooding. It can withstand anoxia temporarily, but not for periods of more than a few days.

Soil anoxia damage plant roots directly by inhibiting cellular respiration. The *critical oxygen pressure* (COP) is the oxygen pressure below which respiration rates decrease as a result of O2 deficiency. The COP for the corn root tip growing in a well-stirred nutrient solution at 25°C is about 20 kilopascals (kPa), or 20% O2 by volume, close to the oxygen concentration in ambient air.

2. Nutrient supply of plant

Unlike **heterotrophic** organisms, which depend for their existence on energy-rich organic molecules previously synthesized by other organisms, plants must survive in an entirely inorganic environment. As **autotrophic** organisms, plants must take in carbon dioxide from the atmosphere and water and mineral nutrients from the soil, and from these simple, inorganic components, make all of the complex molecules of a living organism. Since plants stand at the bottom of the food chain, mineral nutrients assimilated by plants eventually find their way into the matter that makes up all animals, including humans.

Plant nutrition is traditionally treated as two separate topics: organic nutrition and inorganic nutrition. *Organic nutrition* focuses on the production of carbon compounds, specifically the incorporation of carbon, hydrogen, and oxygen via photosynthesis, while *inorganic nutrition* is concerned primarily with the acquisition of mineral elements from the soil. Photosynthesis and the acquisition of mineral ions from the soil are so interdependent, however, that this distinction between organic and inorganic nutrition is more a matter of convenience than real.

2.1. Essential nutrients

Special techniques are used in nutritional studies

To demonstrate that an element is essential requires that plants be grown under experimental conditions in which only the element under investigation is absent. Such conditions are extremely difficult to achieve with plants grown in a complex medium such as soil. In the nineteenth century, several researchers, including Nicolas-Theodore de Saussure, Julius von Sachs, Jean-Baptiste-Joseph-Dieudonne Boussingault, and Wilhelm Knop, approached this problem by growing plants with their roots immersed in a nutrient solution containing only inorganic salts. Their demonstration that plants could grow normally with no soil or organic matter proved unequivocally that plants can fulfill all their needs from only inorganic elements, water, and sunlight.

The technique of growing plants with their roots immersed in a nutrient solution without soil is called *solution culture or hydroponics* (**Figure 1.11**). Successful hydroponic culture requires a large volume of nutrient solution or frequent adjustment of the nutrient solution to prevent nutrient uptake by roots from producing large changes in the nutrient concentrations and pH of the solution. A sufficient supply of oxygen to the root system – also critical – may be achieved by vigorous bubbling of air through the solution. Hydroponics is used in the commercial production of many greenhouse crops, such as tomatoes (*Lycopersicon esculentum*). In another form of hydroponic culture, plant roots lie on the surface of a trough, and nutrient solutions flow in a thin layer along the trough over the roots. This nutrient film growth system ensures that the roots receive an ample supply of oxygen.

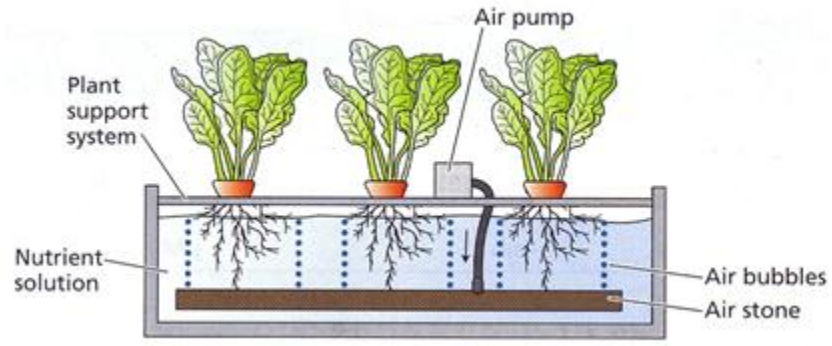


Figure 1.11 Hydroponic growth system: plants are grown in nutrient solution fully saturated with oxygen (*source: Taiz L., Zeiger E., 2010*)

Another alternative, which has sometimes been heralded as the medium of the future for scientific investigations, is to grow the plants *aeroponically*. In this technique plants are grown with their roots suspended in air while being sprayed continuously with a nutrient solution. This approach provides easy manipulation of the gaseous environment around the roots, but it requires higher levels of nutrients than hydroponic culture does to sustain rapid plant growth. For this reason and other technical difficulties, the use of aeroponics is not widespread. An *ebb-and-flow system* is yet another approach to solution culture. In such systems, the nutrient solution periodically rises to immerse plant roots and then recedes, exposing the roots to a moist atmosphere. Like aeroponics, ebb-and-flow systems require higher levels of nutrients than hydroponics or nutrient films.

Nutrient solutions containing only inorganic salts have been used in nutritional studies

Over the years, many formulations have been used for nutrient solutions. Early formulations developed by **Knop** in Germany included only KNO3, Ca(NO3)2, KH2PO4, MgSO4, and an iron salt. At the time, this nutrient solution was believed to contain all the minerals required by plants, but these experiments were carried out with chemicals that were contaminated with other elements that are now known to be essential (such as boron or molybdenum).

A **modified Hoagland solution** contains all the known mineral elements needed for rapid plant growth. The concentrations of these elements are set at the highest possible levels without producing toxicity symptoms or salinity stress, and thus may be several orders of magnitude higher than those found in the soil around plant roots. For example, whereas phosphorus is present in the soil solution at concentrations normally less than 0.06 ppm, here it is offered at 62 ppm. Another important property of the modified Hoagland formulation is that nitrogen is supplied as both ammonium (NH4+) and nitrate (NO3-). Supplying nitrogen in a balanced mixture of cations and anions tends to reduce the rapid rise in the pH of the medium that is commonly observed when the nitrogen is supplied solely as nitrate anion. Even when the pH of the medium is kept neutral, most plants grow better if they have access to both NH4+ and NO3- because absorption and assimilation of the two nitrogen forms promotes cation-anion balances within the plant.

Essential nutrients

Only certain elements have been determined to be essential for plants. An **essential element** is defined as:

- one that is intrinsic component in the structure or metabolism,
- whose absence causes several abnormalities in plant growth, development, or reproduction.

If plants are given these essential elements, as well as water and energy from sunlight, they can synthesize all the compounds they need for normal growth. Hydrogen, carbon, and oxygen are not considered mineral nutrients because they are obtained primarily from water or carbon dioxide.

Essential mineral elements are usually classified as *macronutrients or micronutrients* according to their relative concentrations in plant tissue. In some cases the differences in tissue content between macronutrients and micronutrients are not as great as indicated in the literature. For example, some plant tissues, such as leaf mesophyll, have almost as much iron or manganese as they do sulfur or magnesium. Often elements are present in concentrations greater than the plant's minimum requirements.

The essential elements be classified instead according to their biochemical role and physiological function. Plant nutrients have been divided into four basic groups:

1. Nitrogen and sulfur constitute the first group of essential elements. Plants assimilate these nutrients via biochemical reactions involving oxidation and reduction to form covalent bonds with carbon and create organic compounds.
2. The second group is important in energy storage reactions or in maintaining structural integrity. Elements in this group are often present in plant tissues as phosphate, borate, and silicate esters in which the elemental group is covalently bound to an organic molecule (e.g., sugar phosphate).
3. The third group is present in plant tissue as either free ions dissolved in the plant water or ions electrostatically bound to substances such as the pectic acids present in the plant cell wall. Elements in this group have important roles as enzyme cofactors and in the regulation of osmotic potentials.
4. The fourth group, comprising metals such as iron, has important roles in reactions involving electron transfer.

Some naturally occurring elements, such as aluminum, selenium, and cobalt, that are not essential elements can also accumulate in plant tissues. Aluminum, for example, is not considered to be an essential element, but plants commonly contain from 0.1 to 500 ppm aluminum, and addition of low levels of aluminum to a nutrient solution may stimulate plant growth. Many species in the genera *Astragalus, Xylorhiza, and Stanleya* accumulate selenium, although plants have not been shown to have a specific requirement for this element. Cobalt is part of cobalamin (vitamin B12 and its derivatives), a component of several enzymes in nitrogen-fixing microorganisms. Crop plants normally contain only relatively small amounts of such nonessential elements.

2.2. Nutrient uptake

Soil, roots, and microbes

Soil is complex physically, chemically, and biologically. It is a heterogeneous substance containing solid, liquid, and gaseous phases. All of these phases interact with mineral elements. The inorganic particles of the solid phase provide a reservoir of potassium, calcium, magnesium, and iron. Also associated with this solid phase are organic compounds containing nitrogen, phosphorus, and sulfur, among other elements. The liquid phase of soil constitutes the soil solution, which contains dissolved mineral ions and serves as the medium for ion movement to the root surface. Gases such as oxygen, carbon dioxide, and nitrogen are dissolved in the soil solution, but roots exchange gases with soils predominantly through the air gaps between soil particles.

Negatively charged soil particles affect the adsorption of mineral nutrients

Soil particles, both inorganic and organic, have predominantly negative charges on their surfaces. Many inorganic soil particles are crystal lattices that are tetrahedral arrangements of the cationic forms of aluminum (Al3+) and silicon (Si4+) bound to oxygen atoms, thus forming aluminates and silicates. When cations of lesser charge replace Al3+ and Si4+ within the crystal lattice, these inorganic soil particles become negatively charged. The negative surface charges of organic particles result from the dissociation of hydrogen ions from the carboxylic acid and phenolic groups present in this component of the soil. Most of the world's soil particles, however, are inorganic.

Mineral cations such as ammonium (NH4+) and potassium (K+) adsorb to the negative surface charges of inorganic and organic soil particles. This cation adsorption is an important factor in soil fertility. Mineral cations adsorbed on the surface of soil particles, which are not easily lost when the soil is leached by water, provide a nutrient reserve available to plant roots. Mineral nutrients adsorbed in this way can be replaced by other cations in a process known as *cation exchange.* The degree to which a soil can adsorb and exchange ions is termed its cation exchange capacity (CEC) and is highly dependent on the soil type.

Mineral anions such as nitrate (NO3-) and chloride (Cl-) tend to be repelled by the negative charge on the surface of soil particles and remain dissolved in the soil solution. Thus the *anion exchange* capacity of most agricultural soils is small compared with the cation exchange capacity. Nitrate, in particular, remains mobile in the soil solution, where it is susceptible to leaching by water moving through the soil.

Soil pH affects nutrient availability, excess mineral ions in the soil limit plant growth

Hydrogen ion concentration (pH) is an important property of soils because it affects the growth of plant roots and soil microorganisms. Root growth is generally favored in slightly acidic soils, at pH values between 5.5 and 6.5. Fungi generally predominate in acidic (pH below 7) soils; bacteria become more prevalent in alkaline (pH above 7) soils. Soil pH determines the availability of soil nutrients (**Figure 1.12**). Acidity promotes the weathering of rocks that releases K+, Mg2+, Ca2+, and Mn2+ and increases the solubility of carbonates, sulfates, and phosphates.

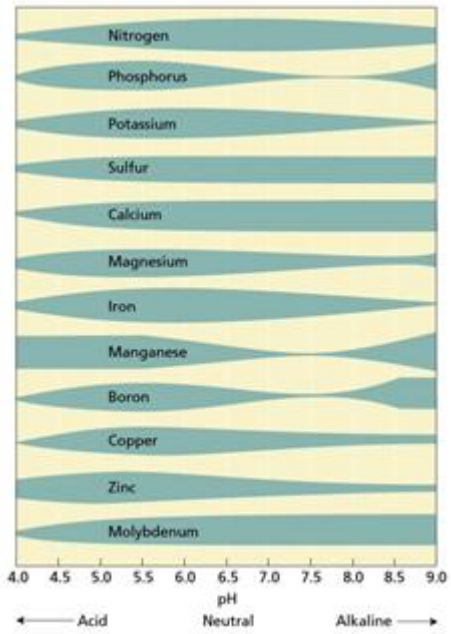


Figure 1.12 Influence of soil pH on the availability of nutrient elements in organic soils (*source: Taiz L., Zeiger E., 2010*)

When excess mineral ions are present in soil, the soil is said to be *saline*, and plant growth may be restricted if these mineral ions reach levels that limit water availability or exceed the adequate zone for a particular nutrient. Sodium chloride and sodium sulfate are the most common salts in saline soils. Excess mineral ions in soils can be a major problem in arid and semiarid regions because rainfall is insufficient to leach them from the soil layers near the surface. Irrigated agriculture fosters soil salinization if the amount of water applied is insufficient to leach the salt below the root zone. Irrigation water can contain 100 to 1000 g of mineral ions per cubic meter, and over a number of growing seasons, high levels of mineral ions may accumulate in the soil. Another important problem with excess mineral ions is the accumulation of heavy metals, e.g., zinc, copper, cobalt, nickel, mercury, lead, cadmium, in the soil, which can cause severe toxicity in plants as well as humans.

Plants develop extensive root system

The ability of plants to obtain both water and mineral nutrients from the soil is related to their capacity to develop an extensive root system. Nonetheless, making observations on root systems is difficult and usually requires special techniques. Plant roots may grow continuously throughout the year. Their proliferation, however, depends on the availability of water and minerals in the immediate microenvironment surrounding the root, the so-called **rhizosphere**. If fertilization and irrigation provide abundant nutrients and water, root growth may not keep pace with shoot growth. Plant growth under such conditions becomes carbohydrate-limited, and a relatively small root system meets the nutrient needs of the whole plant. Indeed, crops under fertilization and irrigation allocate more resources to the shoot and reproductive structures than to roots, and this shift in allocation patterns often results in higher yields.

Within the soil, nutrients can move to the root surface both by bulk flow and by diffusion. In bulk flow, nutrients are carried by water moving through the soil toward the root. The amounts of nutrients provided to the root by bulk flow depend on the rate of water flow through the soil toward the plant, which depends on transpiration rates and on nutrient levels in the soil solution. When both the rate of water flow and the concentrations of nutrients in the soil solution are high, bulk flow can play an important role in nutrient supply. In diffusion, mineral nutrients move from a region of higher concentration to a region of lower concentration. Nutrient uptake by roots lowers the concentrations of nutrients at the root surface, generating concentration gradients in the soil solution surrounding the root.

Roots sense the below ground environment – through gravitropism, thigmotropism, chemotropism, and hydrotropism – to guide their growth toward soil resources. Some of these responses involve auxin. The extent to which roots proliferate within a soil patch varies with nutrient levels (**Figure 1.13**). Root growth is minimal in poor soils because the roots become nutrient-limited. As soil nutrient availability increases, roots proliferate.

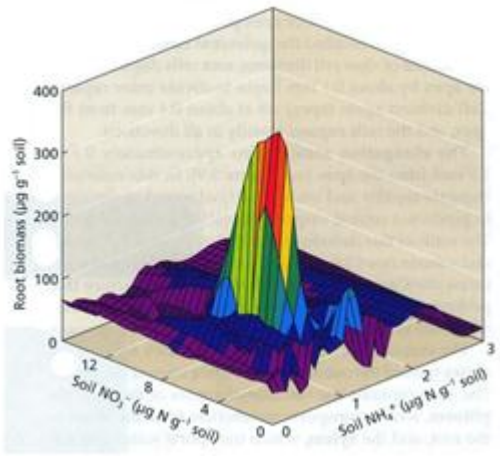


Figure 1.13 Root biomass as a function of extractable soil NH4+ and NO3- (*source: Taiz L., Zeiger E., 2010*)

Mycorrhizal fungi facilitate nutrient uptake by roots

Mycorrhizae (singular *mycorrhiza*) are not unusual; in fact, they are widespread under natural conditions. Much of the world's vegetation appears to have roots associated with mycorrhizal fungi: 83% of dicots, 79% of monocots, and all gymnosperms regularly form mycorrhizal associations. Mycorrhizae are absent from roots in very dry, saline, or flooded soils, or where soil fertility is extreme, either high or low. The host plant provides its associated mycorrhizae with carbohydrates. Mycorrhizal fungi are composed of fine tubular filaments called *hyphae* (singular *hypha*). The mass of hyphae that forms the body of the fungus is called the *mycelium* (plural *mycelia*). There are two major classes of mycorrhizal fungi that are important in terms of mineral nutrient uptake by plants: ectotrophic mycorrhizae and arbuscular mycorrhizae.

Ectotrophic mycorrhizal fungi typically form a thick sheath, or mantle, of mycelium around roots, and some of the mycelium penetrates between the cortical cells (**Figure 1.14**). The cortical cells themselves are not penetrated by the fungal hyphae, but instead are surrounded by a network of hyphae called the *Hartig net*. Often the amount of fungal mycelium is so extensive that its total mass is comparable to that of the roots themselves. The fungal mycelium also extends into the soil. The capacity of the root system to absorb nutrients is improved by the presence of external fungal hyphae because they are much finer than plant roots and can reach beyond the nutrient depletion zone near the roots.

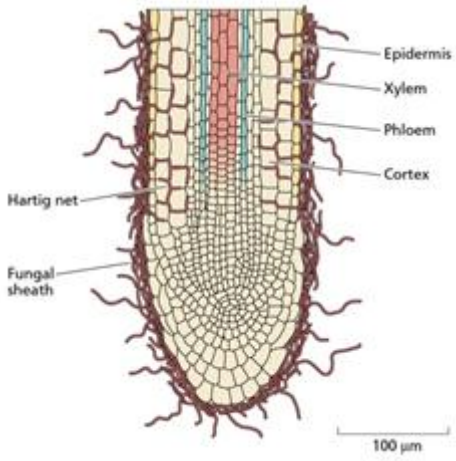


Figure 1.14 Root infected with ectotrophic mycorrhizal fungi (*source: Taiz L., Zeiger E., 2010*)

Unlike the ectotrophic mycorrhizal fungi, *arbuscular mycorrhizal fungi* (previously called vesicular-arbuscular mycorrhizae) do not produce a compact mantle of fungal mycelium around the root. Instead, the hyphae grow in a less dense arrangement, both within the root itself and extending outward from the root into the surrounding soil. After entering the root through either the epidermis or a root hair via a mechanism that has commonalities with the entry of the bacteria responsible for the nitrogen-fixing symbiosis, the hyphae not only extend through the regions between cells, but also penetrate individual cells of the cortex. Within these cells, the hyphae can

form oval structures called vesicles and branched structures called *arbuscules*. The arbuscules appear to be sites of nutrient transfer between the fungus and the host plant.

The association of arbuscular mycorrhizae with plant roots facilitates the uptake of phosphorus, trace metals such as zinc and copper, and water. By extending beyond the depletion zone for phosphorus around the root, the external mycelium improves phosphorus absorption. The external mycelium of ectotrophic mycorrhizae can also absorb phosphate and make it available to plants. Little is known about the mechanism by which the mineral nutrients absorbed by mycorrhizal fungi are transferred to the cells of plant roots.

Symbiotic nitrogen fixation

Biological nitrogen fixation accounts for most of the conversion of atmospheric N2 into ammonium, and thus serves as the key entry point of molecular nitrogen into the biogeochemical cycle of nitrogen. Some bacteria can convert atmospheric nitrogen into ammonium. Most of these nitrogen-fixing prokaryotes live in the soil, generally independent of other organisms. A few form symbiotic associations with higher plants in which the prokaryote directly provides the host plant with fixed nitrogen in exchange for other nutrients and carbohydrates. Such symbioses occur in nodules that form on the roots of the plant and contain the nitrogen-fixing bacteria. The most common type of symbiosis occurs between members of the plant family *Fabaceae (Leguminosae)* and soil bacteria of the genera *Azorhizobium, Bradyrhizobium, Photorhizobium, Rhizobium, and Sinorhizobium* (collectively called *rhizobia*).

Because nitrogen fixation involves the expenditure of large amounts of energy, the *nitrogenase* enzymes that catalyze these reactions have sites that facilitate the high-energy exchange of electrons. Oxygen, being a strong electron acceptor, can damage these sites and irreversibly inactivate nitrogenase, so nitrogen must be fixed under anaerobic conditions. Each of the nitrogen-fixing organisms either functions under natural anaerobic conditions or creates an internal, local anaerobic environment in the presence of oxygen.

Symbiotic nitrogen-fixing prokaryotes dwell within nodules, the special organs of the plant host that enclose the nitrogen-fixing bacteria (**Figure 1.15**). In the case of legumes and actinorhizal plants, the nitrogen-fixing bacteria induce the plant to form root nodules. Grasses can also develop symbiotic relationships with nitrogen-fixing organisms, but in these associations root nodules are not produced. Legumes and actinorhizal plants regulate gas permeability in their nodules, maintaining a level of oxygen within the nodule that can support respiration but is sufficiently low to avoid inactivation of the nitrogenase. Nodules contain an oxygen-binding heme protein called *leghemoglobin*. Leghemoglobin is present in the cytoplasm of infected nodule cells at high concentrations (700 μM in soybean nodules) and gives the nodules a pink color.

Figure 1.15 Root nodules on a common bean (Phaseolus vulgaris) (*source: Taiz L., Zeiger E., 2010*)

The symbiosis between legumes and rhizobia is not obligatory. Legume seedlings germinate without any association with rhizobia, and they may remain unassociated throughout their life cycle. Rhizobia also occur as free-living organisms in the soil. Under nitrogen-limited conditions, however, the symbionts seek each other out through an elaborate exchange of signals. This signaling, the subsequent infection process, and the development of nitrogen-fixing nodules involve specific genes in both the host and the symbionts. Plant genes specific to nodules are called *nodulin (Nod)* genes; rhizobial genes that participate in nodule formation are called

nodulation (nod) genes. The first stage in the formation of the symbiotic relationship between the nitrogen-fixing bacteria and their host is migration of the bacteria toward the roots of the host plant. This migration is a chemotactic response mediated by chemical attractants, especially (iso)flavonoids and betaines, secreted by the roots. These attractants activate the rhizobial NodD protein, which then induces transcription of the other *nod* genes.

Two processes – infection and nodule organogenesis – occur simultaneously during root nodule formation. During the infection process, rhizobia attached to the root hairs release Nod factors that induce a pronounced curling of the root hair cells. The rhizobia become enclosed in the small compartment formed by the *curling*. The cell wall of the root hair degrades in these regions, also in response to Nod factors, allowing the bacterial cells direct access to the outer surface of the plant plasma membrane. The next step is formation of the *infection thread*, an internal tubular extension of the plasma membrane that is produced by the fusion of Golgi-derived membrane vesicles at the site of infection. The thread grows at its tip by the fusion of secretory vesicles to the end of the tube. Deeper into the root cortex, near the xylem, cortical cells dedifferentiate and start dividing, forming a distinct area within the cortex, called a *nodule primordium*, from which the nodule will develop. The infection thread filled with proliferating rhizobia elongates through the root hair and cortical cell layers, in the direction of the nodule primordium. When the infection thread reaches specialized cells within the nodule, its tip fuses with the plasma membrane of the host cell, releasing bacterial cells that are packaged in a membrane derived from the host cell plasma membrane. At first the bacteria continue to divide, and the surrounding membrane increases in surface area to accommodate this growth by fusing with smaller vesicles. Soon thereafter, upon an undetermined signal from the plant, the bacteria stop dividing and begin to enlarge and to differentiate into nitrogen-fixing endosymbiotic organelles called *bacteroids*. The membrane surrounding the bacteroids is called the *peribacteroid* membrane.

Biological nitrogen fixation produces ammonia from molecular nitrogen. The **nitrogenase enzyme complex** – the Fe protein and the *MoFe protein* – catalyzes this reaction. The symbiotic nitrogen-fixing prokaryotes release ammonia that, to avoid toxicity, must be rapidly converted into organic forms in the root nodules before being transported to the shoot via the xylem. Nitrogen-fixing legumes can be classified as amide exporters or ureide exporters, depending on the composition of the xylem sap. Amides (principally the amino acids asparagine or glutamine) are exported by temperate-region legumes, such as pea (*Pisum*), clover (*Trifolium*), broad bean (*Vicia*), and lentil (*Lens*). Ureides are exported by legumes of tropical origin, such as soybean (*Glycine*), common bean (*Phaseolus*). The three major ureides are allantoin, allantoic acid, and citrulline. All three compounds are ultimately released into the xylem and transported to the shoot, where they are rapidly catabolized to ammonium.

Ion transport in roots

Mineral nutrients absorbed by the root are carried to the shoot by the transpiration stream moving through the xylem. Both the initial uptake of nutrients and water and the subsequent movement of these substances from the root surface across the cortex and into the xylem are highly specific, well-regulated processes. Ion transport across the root obeys the same biophysical laws that govern cellular transport.

Solutes move through both apoplast and symplast

In terms of the transport of small molecules, the cell wall is an open lattice of polysaccharides through which mineral nutrients diffuse readily. Because all plant cells are separated by cell walls, ions can diffuse across a tissue (or be carried passively by water flow) entirely through the cell wall space without ever entering a living cell. This continuum of cell walls is called the extracellular space, or **apoplast**. Typically, 5 to 20% of the plant tissue volume is occupied by cell walls. Just as the cell walls form a continuous phase, so do the cytoplasms of neighboring cells, collectively referred to as the **symplast**. Plant cells are interconnected by cytoplasmic bridges called *plasmodesmata*, cylindrical pores 20 to 60 nm in diameter (**Figure 1.16**). Each plasmodesma is lined with plasma membrane and contains a narrow tubule, the *desmotubule*, that is a continuation of the endoplasmic reticulum.

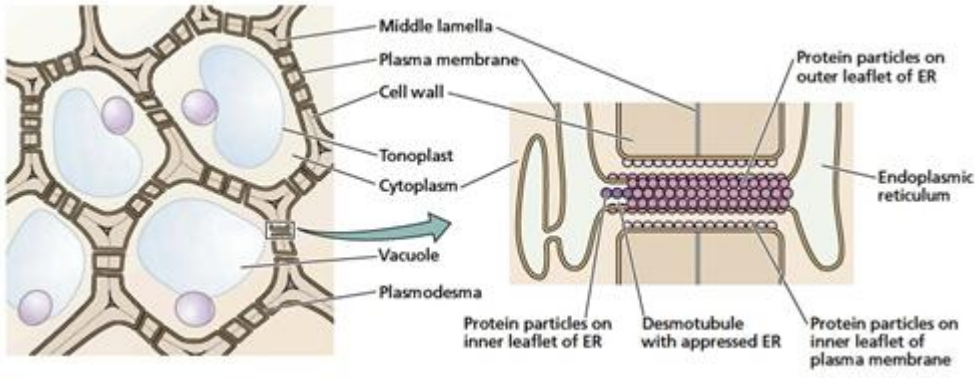


Figure 1.16 Plasmodesmata connect the cytoplasms of neighbouring cells facilitating cell-to-cell communication and solute transport (*source: Taiz L., Zeiger E., 2010*)

Ion absorption by the root is more pronounced in the root hair zone than in the meristem and elongation zones. Cells in the root hair zone have completed their elongation but have not yet begun secondary growth. The root hairs are simply extensions of specific epidermal cells that greatly increase the surface area available for ion absorption. An ion that enters a root may immediately enter the symplast by crossing the plasma membrane of an epidermal cell, or it may enter the apoplast and diffuse between the epidermal cells through the cell walls. From the apoplast of the cortex, an ion (or other solute) may either be transported across the plasma membrane of a cortical cell, thus entering the symplast, or diffuse radially all the way to the endodermis via the apoplast. The apoplast forms a continuous phase from the root surface through the cortex. However, in all cases, ions must enter the symplast before they can enter the stele, because of the presence of the Casparian strip. The Casparian strip is a suberized layer that forms rings around cells of the specialized endodermis and effectively blocks the entry of water and solutes into the stele via the apoplast. Once an ion has entered the stele through the symplastic connections across the endodermis, it continues to diffuse from cell to cell into the xylem. Finally, the ion is released into the apoplast and diffuses into a xylem tracheid or vessel element. The presence of the Casparian strip allows the plant to maintain a higher ion concentration in the xylem than exists in the soil water surrounding the roots.

Xylem parenchima cells participate in xylem loading

Once ions have been taken up into the symplast of the root at the epidermis or cortex, they must be loaded into the tracheids or vessel elements of the stele to be translocated to the shoot. The stele consists of dead tracheary elements and living xylem parenchyma. Because the xylem tracheary elements are dead cells, they lack cytoplasmic continuity with the surrounding xylem parenchyma. To enter the tracheary elements, the ions must exit the symplast by crossing a plasma membrane a second time.

The process whereby ions exit the symplast and enter the conducting cells of the xylem is called *xylem loading*. Xylem parenchyma cells, like other living plant cells, maintain plasma membrane H+-ATPase activity and a negative membrane potential. The plasma membranes of xylem parenchyma cells contain proton pumps, aquaporins, and a variety of ion channels and carriers specialized for influx or efflux. Several types of anion-selective channels have also been identified that participate in unloading of Cl- and NO3- from the xylem parenchyma. Other, less selective ion channels found in the plasma membrane of xylem parenchyma cells are permeable to K+, Na+, and anions.

Passive and active transport

Molecular and ionic movement from one location to another is known as **transport**. Local transport of solutes into or within cells is regulated mainly by membranes. Larger-scale transport between plant organs, or between plant and environment, is also controlled by membrane transport at the cellular level. For example, the transport of sucrose from leaf to root through the phloem, referred to as **translocation**, is driven and regulated by membrane transport into the phloem cells of the leaf and from the phloem to the storage cells of the root.

According to Fick's first law, the movement of molecules by diffusion always proceeds spontaneously, down a gradient of free energy or chemical potential, until equilibrium is reached. The spontaneous "downhill" movement of molecules is termed *passive transport*. At equilibrium, no further net movements of solutes can occur without the application of a driving force. The movement of substances against a gradient of chemical potential, or "uphill", is termed *active transport*. It is not spontaneous, and it requires that work be done on the

system by the application of cellular energy. One common way (but not the only way) of accomplishing this task is to couple transport to the hydrolysis of ATP.

The **chemical potential** for any solute is defined as the sum of the concentration, electric, and hydrostatic potentials (and the chemical potential under standard conditions). The importance of the concept of chemical potential is that it sums all the forces that may act on a molecule to drive net transport. In general, diffusion (passive transport) always moves molecules energetically downhill from areas of higher chemical potential to areas of lower chemical potential. Movement against a chemical-potential gradient is indicative of active transport (**Figure 1.17**). If we take the diffusion of sucrose across a cell membrane as an example, we can accurately approximate the chemical potential of sucrose in any compartment by the concentration term alone.

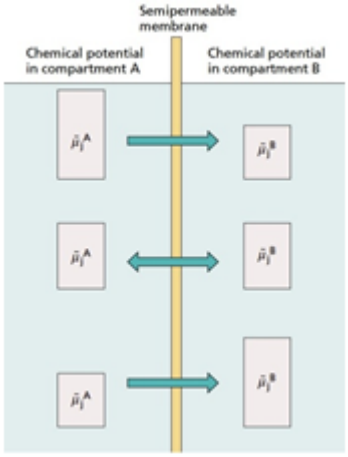


Figure 1.17 Relationship between chemical potential and transport (passive, active) processes (*source: Taiz L., Zeiger E., 2010*)

If the solute carries an electric charge (as does, for example, the potassium ion), the electrical component of the chemical potential must also be considered. Suppose the membrane is permeable to K+ and Cl- rather than to sucrose. K+ ions diffuse in response to both their concentration gradients and any electrical potential difference between the two compartments. Ions can be driven passively against their concentration gradients if an appropriate voltage (electric field) is applied between the two compartments. Because of the importance of electric fields in the biological transport of any charged molecule, an electrochemical potential is exists, and a difference in **electrochemical potential** between the two compartments as well.

If the two KCl solutions in the previous example are separated by a biological membrane, diffusion is complicated by the fact that the ions must move through the membrane as well as across the open solutions. The extent to which a membrane permits the movement of a substance is called **membrane permeability**. Permeability depends on the composition of the membrane as well as on the chemical nature of the solute. When salts diffuse across a membrane, an electrical membrane potential (voltage) can develop. The K+ and Cl- ions will permeate the membrane independently as they diffuse down their respective gradients of electrochemical potential. And unless the membrane is very porous, its permeability to the two ions will differ. As a consequence of these different permeabilities, K+ and Cl- will initially diffuse across the membrane at different rates (**Figure 1.18**). The result is a slight separation of charge, which instantly creates an electrical potential across the membrane. In biological systems, membranes are usually more permeable to K+ than to Cl-. Therefore, K+ will diffuse out of the cell faster than Cl-, causing the cell to develop a negative electric charge with respect to the extracellular medium. A potential that develops as a result of diffusion is called a **diffusion potential**.

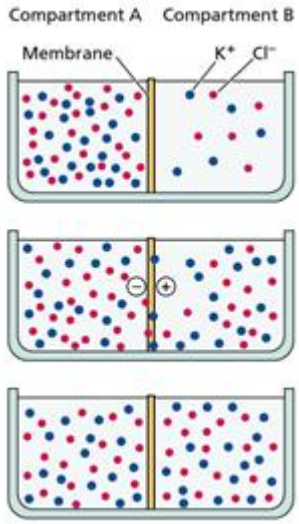


Figure 1.18 Development of a diffusion potential and a charge separation between two compartments separated by a membrane (*source: Taiz L., Zeiger E., 2010*)

The Nernst equation distinguishes between active and passive transport

Because the membrane in the preceding example is permeable to both K+ and Cl- ions, equilibrium will not be reached for either ion until the concentration gradients decrease to zero. However, if the membrane were permeable only to K+, diffusion of K+ would carry charges across the membrane until the membrane potential balanced the concentration gradient. The *Nernst equation* states that at equilibrium, the difference in concentration of an ion between two compartments is balanced by the voltage difference between the compartments. A membrane potential of 59 mV would maintain a tenfold concentration gradient of an ion whose movement across the membrane is driven by passive diffusion.

The concentration of each ion in the external solution bathing the pea root tissue and the measured membrane potential were substituted into the Nernst equation, and a predicted internal concentration was calculated for that ion. The anions NO3-, Cl-, H2PO4-, and SO42- all have higher internal concentrations than predicted, indicating that their uptake is active. The cations Na+, Mg2+, and Ca2+ have lower internal concentrations than predicted; therefore, these ions enter the cell by diffusion down their electrochemical-potential gradients and are then actively exported.

A change in membrane potential caused by an electrogenic pump will change the driving forces for diffusion of all ions that cross the membrane. For example, the outward transport of H+ can create an electrical driving force for the passive diffusion of K+ into the cell.

Membrane transport processes

Artificial membranes made of pure phospholipids have been used extensively to study membrane permeability. Biological and artificial membranes have similar permeabilities to nonpolar molecules and many small polar molecules. On the other hand, biological membranes are much more permeable to ions, to some large polar molecules, such as sugars, and to water than artificial bilayers are. The reason is that, unlike artificial bilayers, biological membranes contain *transport proteins* that facilitate the passage of selected ions and other molecules. The general term transport proteins encompasses three main categories of proteins: **channels, carriers, and pumps** (**Figure 1.19**). Although a particular transport protein is usually highly specific for the kinds of substances it will transport, its specificity is often not absolute. In plants, for example, a K+ transporter in the plasma membrane may transport K+, Rb+, and Na+ with different preferences. In contrast, most K+ transporters are completely ineffective in transporting anions such as Cl- or uncharged solutes such as sucrose.

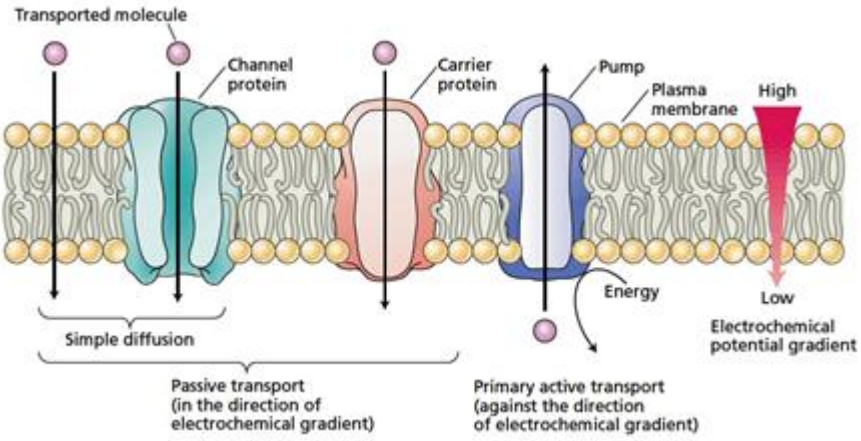


Figure 1.19 Three classes of membrane transport proteins: channels, carriers, and pumps (*source: Taiz L., Zeiger E., 2010*)

Channels enhance diffusion across membranes

Channels are transmembrane proteins that function as selective pores through which molecules or ions can diffuse across the membrane. The size of a pore and the density and nature of the surface charges on its interior lining determine its transport specificity. Transport through channels is always passive, and because the specificity of transport depends on pore size and electric charge more than on selective binding, channel transport is limited mainly to ions or water. As long as the channel pore is open, solutes that can penetrate the pore diffuse through it extremely rapidly: about 108 ions per second through each channel protein. Channel pores are not open all the time, however. Channel proteins have structures called *gates* that open and close the pore in response to external signals. Signals that can regulate channel activity include membrane potential changes, ligands, hormones, light, and posttranslational modifications such as phosphorylation. Individual ion channels can be studied in detail by an electrophysiological technique called *patch clamping*, which can detect the electrical current carried by ions diffusing through a single open channel or a collection of channels.

Carriers bind and transport specific substances

Unlike channels, **carrier proteins** do not have pores that extend completely across the membrane. In transport mediated by a carrier, the substance being transported is initially bound to a specific site on the carrier protein. This requirement for binding allows carriers to be highly selective for a particular substrate to be transported. Carriers therefore specialize in the transport of specific ions or organic metabolites. Binding causes a conformational change in the protein, which exposes the substance to the solution on the other side of the membrane. Transport is complete when the substance dissociates from the carrier's binding site. Because a conformational change in the protein is required to transport an individual molecule or ion, the rate of transport by a carrier is many orders of magnitude slower than that through a channel. Carrier-mediated transport (unlike transport through channels) can be either passive transport or secondary active transport. Passive transport via a carrier is sometimes called *facilitated diffusion*, although it resembles diffusion only in that it transports substances down their gradient of electrochemical potential, without an additional input of energy.

Primary active transport, called pumps, requires direct energy source

To carry out active transport, a carrier must couple the energetically uphill transport of a solute with another, energy-releasing event so that the overall free-energy change is negative. *Primary active transport* is coupled directly to a source of energy, such as ATP hydrolysis, an oxidation-reduction reaction (as in the electron transport chain of mitochondria and chloroplasts), or the absorption of light by the carrier protein (such as bacteriorhodopsin in halobacteria). Membrane proteins that carry out primary active transport are called **pumps**. Most pumps transport ions, such as H+ or Ca2+. However, pumps belonging to the ATP-binding cassette (ABC) family of transporters can carry large organic molecules. For the plasma membranes of plants, fungi, and bacteria, as well as for plant tonoplasts and other plant and animal endomembranes, H+ is the principal ion that is electrogenically pumped across the membrane. The plasma membrane H+-ATPase generates the gradient of electrochemical potential of H+ across the plasma membrane, while the vacuolar H+-ATPase and the H+-pyrophosphatase (H+-PPase) electrogenically pump protons into the lumen of the vacuole and the Golgi cisternae.

Secondary active transport uses stored energy

In plant plasma membranes, the most prominent pumps are those for H+ and Ca2+, and the direction of pumping is outward from the cytosol to the extracellular space. Another mechanism is needed to drive the active uptake of mineral nutrients such as NO3-, SO42-, and PO43-; the uptake of amino acids, peptides, and sucrose; and the export of Na+, which at high concentrations is toxic to plant cells. The other important way that solutes are actively transported across a membrane against their gradient of electrochemical potential is by coupling the uphill transport of one solute to the downhill transport of another. This type of carrier-mediated cotransport is termed *secondary active transport*. Secondary active transport is driven indirectly by pumps. The gradient of electrochemical potential for H+ referred to as **proton motive force (PMF)**, represents stored free energy in the form of the H+ gradient. The proton motive force generated by electrogenic H+ transport is used in secondary active transport to drive the transport of many other substances against their gradients of electrochemical potential. There are two types of secondary active transport: **symport and antiport**. Symport is the transport process when two substances move in the same direction through the membrane. Antiport refers to coupled transport in which the energetically downhill movement of protons drives the active (energetically uphill) transport of a solute in the opposite direction. In both types of secondary transport, the ion or solute being transported simultaneously with the protons is moving against its gradient of electrochemical potential, so its transport is active.

Cations are transported by both cation channels and cation carriers

Transport across biological membrane is energized by one primary active transport system coupled to ATP hydrolysis. The transport of one ionic species – for example, H+ – generates an ion gradient and an electrochemical potential. Many other ions or organic substrates can then be transported by a variety of secondary active transport proteins, which energize the transport of their substrates by simultaneously carrying one or two H+ down their energy gradient. Thus protons circulate across the membrane, outward through the primary active transport proteins and back into the cell through the secondary active transport proteins.

The relative contributions of each type of cation transport mechanism differ depending on the membrane, cell type, and biological phenomenon under investigation. Some of the *cation channels* are highly selective for specific ionic species, such as potassium ions. Others allow passage of a variety of cations, sometimes including Na+, even though this ion is toxic when overaccumulated.

A variety of *carriers* also move cations into plant cells. There are two families of transporters that specialize in K+ transport across plant membranes: the KUP/HAK/KT family and the HKTs. A third family, the cation-H+ antiporters (CPAs), mediates electroneutral exchange of H+ and other cations, including K+ in some cases.

Anion transporters have been identified

Nitrate (NO3-), chloride (Cl-), sulfate (SO4-), and phosphate (PO43-) are the major inorganic ions in plant cells, and malate2- is the major organic anion. The free-energy gradient for all of these anions is in the direction of passive efflux. Several types of plant anion channels have been characterized by electrophysiological techniques, and most anion channels appear to be permeable to a variety of anions. In contrast to the relative lack of specificity of anion channels, anion carriers that mediate the energetically uphill transport of anions into plant cells exhibit selectivity for particular anions. Plants have transporters for various organic anions, such as malate and citrate.

Phosphate availability in the soil solution often limits plant growth. Phosphate-H+ symporters with lower affinity for phosphate have also been identified in plants and have been localized to membranes of intracellular organelles such as plastids and mitochondria. Another group of phosphate transporters, the phosphate translocators, are located in the inner plastid membrane, where, in exchange for uptake of inorganic phosphate, they function to release phosphorylated carbon compounds derived from photosynthesis to the cytosol.

Aquaporins forms water channels in membranes

Water channels, or **aquaporins**, are a class of proteins that are relatively abundant in plant membranes. Many aquaporins do not result in ion currents when expressed in oocytes, consistent with a lack of ion transport activity, but when the osmolarity of the external medium is reduced, expression of these proteins results in swelling and bursting of the oocytes. The bursting results from rapid influx of water across the oocyte plasma membrane, which normally has very low permeability to water. These results confirm that aquaporins form water channels in membranes. Some aquaporin proteins also transport uncharged solutes (e.g., NH3), and there

is some evidence that aquaporins act as conduits for carbon dioxide uptake into plant cells. Aquaporin activity is regulated by phosphorylation as well as by pH, calcium concentration, heteromerization, and reactive oxygen species.

Plasma membrane H+-ATPases are important for the regulation of cytoplasmic pH and for the control of cell turgor

The outward active transport of protons across the plasma membrane creates gradients of pH and electrical potential that drive the transport of many other substances (ions and uncharged solutes) through the various secondary active transport proteins. H+-ATPase activity is also important for the regulation of cytoplasmic pH and for the control of cell turgor, which drives organ (leaf and flower) movement, stomatal opening, and cell growth. Plant plasma membrane H+-ATPases are encoded by a family of about a dozen genes. In general, H+-ATPase expression is high in cells with key functions in nutrient movement, including root endodermal cells and cells involved in nutrient uptake from the apoplast that surrounds the developing seed. Like other enzymes, the plasma membrane H+-ATPase is regulated by the concentration of substrate (ATP), pH, temperature, and other factors. In addition, H+-ATPase molecules can be reversibly activated or deactivated by specific signals, such as light, hormones, or pathogen attack.

Plant cells increase their size primarily by taking up water into a large central vacuole. Therefore, the osmotic pressure of the vacuole must be kept sufficiently high for water to enter from the cytoplasm. The tonoplast regulates the traffic of ions and metabolites between the cytosol and the vacuole, just as the plasma membrane regulates their uptake into the cell. The *vacuolar H+-ATPase* (also called *V-ATPase*) differs both structurally and functionally from the plasma membrane H+-ATPase.

2.3. Solute transport

Phloem translocation

Phloem translocation moves the products of photosynthesis from mature leaves to areas of growth and storage. It also transmits chemical signals and redistributes ions and other substances throughout the plant body.

Pathways of translocation

An analysis of phloem exudate provides more direct evidence in support of the conclusion that photoassimilates are translocated through the phloem. Unfortunately, phloem tissue does not lend itself to analysis as easily as xylem tissue does. This is because the translocating elements in the phloem are, unlike xylem vessels and tracheids, living cells when functional. The distinguishing feature of phloem tissue is the conducting cell called the *sieve element*. Also known as a *sieve tube*, the sieve element is an elongated rank of individual cells, called sieve-tube members, arranged end-to-end. Unlike xylem tracheary elements, phloem sieve elements lack rigid walls and contain living protoplasts when mature and functional. The protoplasts of contiguous sieve elements are interconnected through specialized *sieve areas* in adjacent walls. Where the pores of the sieve area are relatively large and are found grouped in a specific area, they are known as *sieve plates*. Sieve plates are typically found in the end walls of sieve-tube members and provide a high degree of protoplasmic continuity between consecutive sieve-tube members. Additional pores are found in sieve areas located in lateral walls. In addition to sieve elements, phloem tissue also contains a variety of parenchyma cells. Some of these cells are intimately associated with sieve-tube members and for this reason are called *companion cells*. The interdependence of the sieve-tube member and companion cells is reflected in their lifetime – the companion cell remains alive only so long as the sieve-tube member continues to function. Companion cells are believed to provide metabolic support for the sieve-tube member.

Materials translocated in the phloem

Phloem sap can be collected from aphid stylets or, alternatively, from some plants by simply making an incision into the bark. If done carefully, to avoid cutting into the underlying xylem, the incision opens the sieve tubes and a relatively pure exudate can be collected in very small microcapillary tubes for subsequent analysis. As might be expected, the chemical composition of phloem exudate is highly variable. It depends on the species, age, and physiological condition of the tissue sampled. Even for a particular sample under uniform conditions, there may be wide variations in the concentrations of particular components between subsequent samples. For example, an analysis of phloem exudate from stems of actively growing castor bean (*Ricinus communis*) shows that the exudate contains sugars, protein, amino acids, the organic acid malate, and a variety of inorganic anions and cations. The inorganic anions include phosphate, sulphate, and chloride – nitrate is conspicuously absent –

while the predominant cation is potassium. Some plant hormones (auxin, cytokinin, and gibberellin) were also detected, but at very low concentrations. The principal constituent of phloem exudate in most species is *sugar*. In castor bean it is sucrose, which comprises approximately 80 percent of the dry matter. A survey of over 500 species representing approximately 100 dicotyledonous families confirms that sucrose is almost universal as the dominant sugar in the phloem stream.

It is interesting to speculate on why sucrose is the preferred vehicle for long-distance translocation of photoassimilate. One possibility is that sucrose, a disaccharide, and its related oligosaccharides are nonreducing sugars. On the other hand, all monosaccharides, including glucose and fructose, are reducing sugars. Reducing sugars have a free aldehyde or ketone group that is capable of reducing mild oxidizing agents. Some oligosaccharides, such as sucrose, are nonreducing sugars because the acetal link between the subunits is stable and nonreactive in alkaline solution. The exclusive use of nonreducing sugars in the translocation of photoassimilate may be related to this greater chemical stability.

The pressure-flow model, a passive mechanism for phloem transport

Any comprehensive theory for phloem translocation must take into account a number of factors. These include: (1) the structure of sieve elements, including the presence of active cytoplasm, P-protein (phloem protein), and resistances imposed by sieve plates; (2) observed rapid rates of translocation (50 to 250 cm hr-1) over long distances; (3) translocation in different directions at the same time; (4) the initial transfer of assimilate from leaf mesophyll cells into sieve elements of the leaf minor veins (called *phloem loading*); and (5) final transfer of assimilate out of the sieve elements into target cells (called *phloem unloading*).

The most credible and generally accepted model for phloem translocation is one of the earliest. Originally proposed by E. Münch in 1930 but modified by a series of investigators since, the *pressure-flow hypothesis* remains the simplest model and continues to earn widespread support among plant physiologists. The pressure-flow mechanism is based on the mass transfer of solute from source to sink along a hydrostatic (turgor) pressure gradient. Translocation of solute in the phloem is closely linked to the flow of water in the transpiration stream and a continuous recirculation of water in the plant. The principle of pressure flow can be easily demonstrated in the laboratory by connecting two osmometers (**Figure 1.20**).

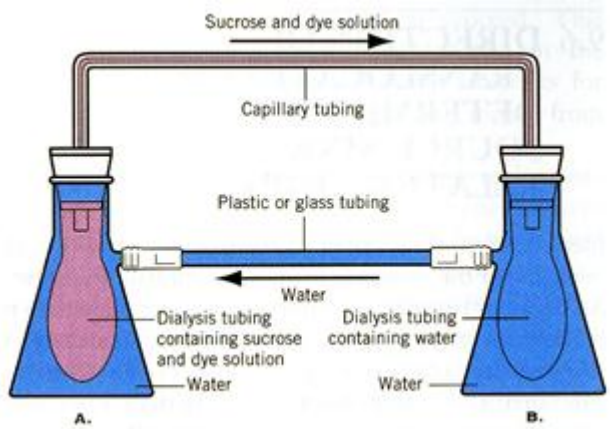


Figure 1.20 A physical model of the pressure-flow hypothesis for translocation in the phloem (*source: Taiz L., Zeiger E., 2010*)

Assimilate translocation begins with the loading of sugars into sieve elements at the source. Typically, loading would occur in the minor veins of a leaf, close to a photosynthetic mesophyll or bundle-sheath cell. The increased solute concentration in the sieve element lowers its water potential and, consequently, is accompanied by the osmotic uptake of water from the nearby xylem. This establishes a higher turgor or hydrostatic pressure in the sieve element at the source end. At the same time, sugar is unloaded at the sink end – a root or stem storage cell, for example. The hydrostatic pressure at the sink end is lowered as water leaves the sieve elements and returns to the xylem. So long as assimilates continue to be loaded at the source and unloaded at the sink, this pressure differential will be maintained, water will continue to move in at the source and out at the sink, and assimilate will be carried passively along. According to the pressure-flow hypothesis, solute translocation in the phloem is fundamentally a passive process; that is, translocation requires no direct input of metabolic energy to make it function.

Photosynthate distribution: allocation and partitioning

The photosynthetic rate determines the total amount of fixed carbon available to the leaf. However, the amount of fixed carbon available for translocation depends on subsequent metabolic events. The regulation of the distribution of fixed carbon into various metabolic pathways is termed **allocation**. The vascular bundles in a plant form a system of "pipes" that can direct the flow of photosynthates to various sinks: young leaves, stems, roots, fruits, or seeds. However, the vascular system is often highly interconnected, forming an open network that allows source leaves to communicate with multiple sinks. Under these conditions, what determines the volume of flow to any given sink? The differential distribution of photosynthates within the plant is termed **partitioning**.

The carbon fixed in a source cell can be used for storage, metabolism, and transport:

- *Synthesis of storage compounds*. Starch is synthesized and stored within chloroplasts and, in most species, is the primary storage form that is mobilized for translocation during the night. Plants that store carbon primarily as starch are called "starch storers".

- *Metabolic utilization*. Fixed carbon can be utilized within various compartments of the photosynthesizing cell to meet the energy needs of the cell or to provide carbon skeletons for the synthesis of other compounds required by the cell.

- *Synthesis of transport compounds*. Fixed carbon can be incorporated into transport sugars for export to various sink tissues. A portion of the transport sugar can also be stored temporarily in the vacuole.

Transport of signaling molecules

Besides its major function in the long-distance transport of photosynthate, the phloem is also a conduit for the transport of signaling molecules from one part of the organism to another. Such long-distance signals coordinate the activities of sources and sinks and regulate plant growth and development. The signals between sources and sinks might be physical or chemical. *Physical signals* such as turgor change could be transmitted rapidly via the interconnecting system of sieve elements. Molecules traditionally considered to be *chemical signals*, such as proteins and plant hormones, are found in the phloem sap, as are mRNAs and small RNAs, which have more recently been added to the list of signal molecules. The translocated carbohydrates themselves may also act as signals.

Shoots produce growth regulators such as auxin, which can be rapidly transported to the roots via the phloem, and roots produce cytokinins, which move to the shoots through the xylem. Gibberellins (GA) and abscisic acid (ABA) are also transported throughout the plant in the vascular system. Plant hormones play a role in regulating source-sink relationships. They affect photosynthate partitioning in part by controlling sink growth, leaf senescence, and other developmental processes. Plant defense responses against herbivores and pathogens can also change allocation and partitioning of photoassimilates, with plant defense hormones such as jasmonic acid mediating the responses.

It has long been known that viruses can move in the phloem, traveling as complexes of proteins and nucleic acids or as intact virus particles. More recently, endogenous RNA molecules and proteins have been found in phloem sap, and at least some of these can function as signal molecules or generate phloem-mobile signals. To be assigned a signaling role in plants, a macromolecule must meet a number of significant criteria:

- The macromolecule must move from source to sink in the phloem.

- The macromolecule must be able to leave the sieve element-companion cell complex in sink tissues. Alternatively, the macromolecule might trigger the formation of a second signal that transmits information to the sink tissues surrounding the phloem; that is, it might initiate a signal cascade.

- Perhaps most important, the macromolecule must be able to modify the functions of specific cells in the sink.

Plasmodesmata have been implicated in nearly every aspect of phloem translocation, from loading to long-distance transport (pores in sieve areas and sieve plates are modified plasmodesmata) to allocation and partitioning. The mechanism of plasmodesmatal transport (called trafficking) can be either passive (non targeted) or selective and regulated.

2.4. Nutritional deficiencies

Requirements for mineral elements change during the growth and development of a plant. In crop plants, nutrient levels at certain stages of growth influence the yield of the economically important tissues (tuber, grain, and so on). To optimize yields, farmers use analyses of nutrient levels in soil and in plant tissue to determine fertilizer schedules.

Analysis of plant tissues reveals mineral deficiencies

Soil analysis is the chemical determination of the nutrient content in a soil sample from the root zone. Both the chemistry and the biology of soils are complex, and the results of soil analyses vary with sampling methods, storage conditions for the samples, and nutrient extraction techniques. Perhaps more important is that a particular soil analysis reflects the levels of nutrients potentially available to the plant roots from the soil, but soil analysis does not tell us how much of a particular mineral nutrient the plant actually needs or is able to absorb. This additional information is best determined by plant tissue analysis.

Proper use of **plant tissue analysis** requires an understanding of the relationship between plant growth (or yield) and the concentration of a nutrient in plant tissue samples (**Figure 1.21**). Three zones (deficiency, adequate, and toxic) are identified in the response of growth to increasing tissue concentrations of a nutrient. When the nutrient concentration in a tissue sample is low, growth is reduced. In this *deficiency zone* of the curve, an increase in nutrient availability is directly related to an increase in growth or yield. As nutrient availability continues to increase, a point is reached at which further addition of the nutrient is no longer related to increases in growth or yield, but is reflected in increased tissue concentrations. This region of the curve is called the *adequate zone*. The point of transition between the deficiency and adequate zones of the curve reveals the *critical concentration* of the nutrient (**Figure 1.21**), which may be defined as the minimum tissue content of the nutrient that is correlated with maximal growth or yield. As the nutrient concentration of the tissue increases beyond the adequate zone, growth or yield declines because of toxicity (this region of the curve is the *toxic zone*).

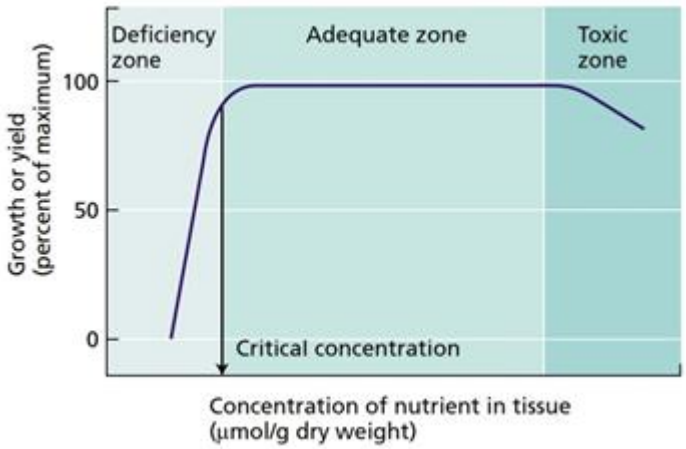


Figure 1.21 Relationship between yield (or growth) and the nutrient content of the plant tissue (*source: Taiz L., Zeiger E., 2010*)

Because agricultural soils are often limited in the elements nitrogen, phosphorus, and potassium, many farmers routinely take into account, at a minimum, growth or yield responses for these elements. If a nutrient deficiency is suspected, steps are taken to correct the deficiency before it reduces growth or yield. Plant analysis has proved useful in establishing fertilizer schedules that sustain yields and ensure the food quality of many crops.

Some essential elements can be recycled from older to younger leaves, others are relatively immobile

An important clue in relating an acute deficiency symptom to a particular essential element is the extent to which an element can be recycled from older to younger leaves. Some elements, such as nitrogen, phosphorus, and potassium, can readily move from leaf to leaf; others, such as boron, iron, and calcium, are relatively immobile in most plant species. If an essential element is *mobile*, deficiency symptoms tend to appear first in older leaves. Deficiency of an *immobile* essential element becomes evident first in younger leaves. Although the precise mechanisms of nutrient mobilization are not well understood, plant hormones such as cytokinins appear to be involved.

Inadequate supply of an essential element is manifested by characteristic deficiency symptoms

Mineral deficiencies disrupt plant metabolism and function. In hydroponic culture, withholding of an essential element can be readily correlated with a given set of symptoms.

Diagnosis of soil-grown plants can be more complex for the following reasons:

- deficiencies of several elements may occur simultaneously in different plant tissues,
- deficiencies or excessive amounts of one element may induce deficiencies or excessive accumulations of another,
- some virus-induced plant diseases may produce symptoms similar to those of nutrient deficiencies.

Nutrient deficiency symptoms in a plant are the expression of metabolic disorders resulting from the insufficient supply of an essential element. These disorders are related to the roles played by essential elements in normal plant metabolism and function.

Although each essential element participates in many different metabolic reactions, some general statements about the functions of essential elements in plant metabolism are possible. In general, the essential elements function in plant structure, metabolism, and cellular osmoregulation. More specific roles may be related to the ability of divalent cations such as calcium or magnesium to modify the permeability of plant membranes. In addition, research continues to reveal specific roles for these elements in plant metabolism.

In the discussion that follows, we will describe the specific deficiency symptoms and functional roles of the mineral essential elements. According to the four basic group of essential elements, deficiency symptoms of plant minerals can be classified as:

- Group 1: deficiencies in mineral nutrients that are part of carbon compounds (N, S),
- Group 2: deficiencies in mineral nutrients that are important in energy storage or structural integrity (P, Si, B),
- Group 3: deficiencies in mineral nutrients that remain in ionic form (K, Ca, Mg, Cl, Mn, Na),
- Group 4: deficiencies in mineral nutrients that are involved in redox reactions (Fe, Zn, Cu, Ni, Mo).

Deficiencies in mineral nutrients that are part of carbon compounds (N, S)

This first group consists of nitrogen and sulfur. Nitrogen availability in soils limits plant productivity in most natural and agricultural ecosystems. By contrast, soils generally contain sulfur in excess. Some of the most energy-intensive reactions in life convert the highly oxidized, inorganic forms, such as nitrate and sulfate, that roots absorb from the soil into the highly reduced forms found in organic compounds such as amino acids within plants.

Nitrogen is the mineral element that plants require in the greatest amounts. It serves as a constituent of many plant cell components, including amino acids, proteins, and nucleic acids. Therefore nitrogen deficiency rapidly inhibits plant growth. If such a deficiency persists, most species show *chlorosis* (yellowing of the leaves), especially in the older leaves near the base of the plant. Under severe nitrogen deficiency, these leaves become completely yellow (or tan) and fall off the plant. Younger leaves may not show these symptoms initially because nitrogen can be mobilized from older leaves. Carbohydrates not used in nitrogen metabolism may also be used in anthocyanin synthesis, leading to accumulation of that pigment. This condition is revealed as a purple coloration in leaves, petioles, and stems of nitrogen-deficient plants of some species, such as tomato and certain varieties of maize (*Zea mays*).

Sulfur is found in amino acids (cystine, cysteine, and methionine) and is a constituent of several coenzymes and vitamins, such as coenzyme A, S-adenosylmethionine, biotin, vitamin B1, and pantothenic acid, which are essential for metabolism. Many of the symptoms of sulfur deficiency are similar to those of nitrogen deficiency, including chlorosis, stunting of growth, and anthocyanin accumulation. The chlorosis caused by sulfur deficiency, however, generally arises initially in mature and young leaves, rather than in old leaves as in nitrogen deficiency, because sulfur, unlike nitrogen, is not easily remobilized to the younger leaves in most species.

Deficiencies in mineral nutrients that are important in energy storage or structural integrity (P, Si, B)

This group consists of phosphorus, silicon, and boron. Phosphorus and silicon are found at concentrations within plant tissue that warrant their classification as macronutrients, whereas boron is much less abundant and is considered a micronutrient.

Phosphorus (as phosphate, PO43-) is an integral component of important compounds of plant cells, including the sugar-phosphate intermediates of respiration and photosynthesis as well as the phospholipids that make up plant membranes. It is also a component of nucleotides used in plant energy metabolism (such as ATP) and in DNA and RNA. Characteristic symptoms of phosphorus deficiency include stunted growth in young plants and a dark green coloration of the leaves. As in nitrogen deficiency, some species may produce excess anthocyanins, giving the leaves a slight purple coloration.

Plants deficient in **silicon** are more susceptible to lodging (falling over) and fungal infection. Silicon is deposited primarily in the endoplasmic reticulum, cell walls, and intercellular spaces as hydrated, amorphous silica ($SiO_2 \bullet nH_2O$). It also forms complexes with polyphenols and thus serves as an alternative to lignin in the reinforcement of cell walls.

Boron-deficient plants may exhibit a wide variety of symptoms, depending on the species and the age of the plant. A characteristic symptom is black necrosis of young leaves and terminal buds. The necrosis of the young leaves occurs primarily at the base of the leaf blade. Apical dominance may also be lost, causing the plant to become highly branched. Structures such as the fruits, fleshy roots, and tubers may exhibit necrosis or abnormalities related to the breakdown of internal tissues.

Deficiencies in mineral nutrients that remain in ionic form (K, Ca, Mg, Cl, Mn, Na)

This group includes some of the most familiar mineral elements: the macronutrients potassium, calcium, and magnesium and the micronutrients chlorine, manganese, and sodium. These elements may be found as ions in solution in the cytosol or vacuoles, or they may be bound electrostatically or as ligands to larger, carbon-containing compounds.

Potassium, present within plants as the cation K^+, plays an important role in regulation of the osmotic potential of plant cells. It also activates many enzymes involved in respiration and photosynthesis. The first observable symptom of potassium deficiency is mottled or marginal chlorosis, which then develops into necrosis primarily at the leaf tips, at the margins, and between veins. In many monocots, these necrotic lesions may initially form at the leaf tips and margins and then extend toward the leaf base. Because potassium can be mobilized to the younger leaves, these symptoms appear initially on the more mature leaves toward the base of the plant.

Calcium ions (Ca^{2+}) are used in the synthesis of new cell walls. It is required for the normal functioning of plant membranes and has been implicated as a second messenger for various plant responses to both environmental and hormonal signals. Characteristic symptoms of calcium deficiency include necrosis of young meristematic regions such as the tips of roots or young leaves, where cell division and cell wall formation are most rapid. Necrosis in slowly growing plants may be preceded by a general chlorosis and downward hooking of young leaves. The root system of a calcium-deficient plant may appear brownish, short, and highly branched.

In plant cells, **magnesium** ions (Mg^{2+}) have a specific role in the activation of enzymes involved in respiration, photosynthesis, and the synthesis of DNA and RNA. Magnesium is also a part of the ring structure of the chlorophyll molecule. A characteristic symptom of magnesium deficiency is chlorosis between the leaf veins, occurring first in older leaves because of the mobility of this cation.

The element **chlorine** is found in plants as the chloride ion (Cl^-). It is required for the water-splitting reaction of photosynthesis through which oxygen is produced. Plants deficient in chlorine develop wilting of the leaf tips followed by general leaf chlorosis and necrosis. The leaves may also exhibit reduced growth. Roots of chlorine-deficient plants may appear stunted and thickened near the root tips. Chloride ions are highly soluble and are generally available in soils. Therefore chlorine deficiency is only rarely observed in plants grown in native or agricultural habitats.

Manganese ions (Mn^{2+}) activate several enzymes in plant cells. In particular, decarboxylases and dehydrogenases involved in the citric acid (Krebs) cycle are specifically activated by manganese. The best-defined function of manganese is in the photosynthetic reaction through which oxygen (O_2) is produced from water. The major symptom of manganese deficiency is intervenous chlorosis associated with the development of small necrotic spots.

Most species utilizing the C4 and crassulacean acid metabolism (CAM) pathways of carbon fixation require **sodium** ions (Na^+). In these plants, sodium appears vital for regenerating phosphoenolpyruvate, the substrate for the first carboxylation in the C4 and CAM pathways. Under sodium deficiency, these plants exhibit chlorosis and necrosis, or even fail to form flowers. Many C3 species also benefit from exposure to low levels of sodium ions.

Deficiencies in mineral nutrients that are involved in redox reactions (Fe, Zn, Cu, Ni, Mo)

This group of five micronutrients consists of the metals iron, zinc, copper, nickel, and molybdenum. All of these can undergo reversible oxidations and reductions (e.g., $Fe^{2+} \leftrightarrow Fe^{3+}$) and have important roles in electron transfer and energy transformation. They are usually found in association with larger molecules such as cytochromes, chlorophyll, and proteins (usually enzymes).

Iron has an important role as a component of enzymes involved in the transfer of electrons (redox reactions), such as cytochromes. As in magnesium deficiency, a characteristic symptom of iron deficiency is intervenous chlorosis. These symptoms, however, appear initially on younger leaves because iron, unlike magnesium, cannot be readily mobilized from older leaves. Under conditions of extreme or prolonged deficiency, the veins may also become chlorotic, causing the whole leaf to turn white.

Many enzymes require **zinc** ions (Zn^{2+}) for their activity, and zinc may be required for chlorophyll biosynthesis in some plants. Zinc deficiency is characterized by a reduction in internodal growth, and as a result plants display a rosette habit of growth in which the leaves form a circular cluster radiating at or close to the ground. The leaves may also be small and distorted, with leaf margins having a puckered appearance. These symptoms may result from loss of the capacity to produce sufficient amounts of the auxin indole-3-acetic acid (IAA).

Like iron, **copper** is associated with enzymes involved in redox reactions, through which it is reversibly oxidized from Cu^+ to Cu^{2+} An example of such an enzyme is plastocyanin, which is involved in electron transfer during the light reactions of photosynthesis. The initial symptom of copper deficiency is the production of dark green leaves, which may contain necrotic spots. The necrotic spots appear first at the tips of young leaves and then extend toward the leaf base along the margins.

Urease is the only known **nickel**-containing (Ni^{2+}) enzyme in higher plants, although nitrogen-fixing microorganisms require nickel (Ni^+ through Ni^{4+}) for the enzyme that reprocesses some of the hydrogen gas generated during fixation (hydrogen uptake hydrogenase). Nickel-deficient plants accumulate urea in their leaves and consequently show leaf tip necrosis.

Molybdenum ions (Mo^{4+} through Mo^{6+}) are components of several enzymes, including nitrate reductase and nitrogenase. The first indication of a molybdenum deficiency is general chlorosis between veins and necrosis of older leaves. In some plants, such as cauliflower or broccoli, the leaves may not become necrotic, but instead may appear twisted and subsequently die. Flower formation may be prevented, or the flowers may abscise prematurely.

Treating nutritional deficiencies

Many traditional and subsistence farming practices promote the recycling of mineral elements. The main losses of nutrients from such agricultural systems ensue from leaching that carries dissolved ions, especially nitrate, away with drainage water. In the high-production agricultural systems of industrialized countries, a large proportion of crop biomass leaves the area of cultivation, and returning crop residues to the land where the crop was produced becomes difficult at best. This unidirectional removal of nutrients from agricultural soils make it important to restore the lost nutrients to these soil through the addition of fertilizers. Most *chemical fertilizers* contain inorganic salts of the macronutrients nitrogen, phosphorus, and potassium. Fertilizers that contain only one of these three nutrients are termed *straight fertilizers*, like superphosphate, ammonium nitrate. Fertilizers that contain two or more mineral nutrients are called compound fertilizers or *mixed fertilizers*, and the numbers on the package label, such as "10-14-10", refer to the percentages of N, P as P_2O_5 and K as K_2O, respectively, in the fertilizer. With long-term agricultural production, consumption of micronutrients can reach a point at which they, too, must be added to the soil as fertilizers.

Organic fertilizers, in contrast to chemical fertilizers, originate from the residues of plant or animal life or from natural rock deposits. Before crop plants can acquire the nutrient elements from these residues, the organic compounds must be broken down, usually by the action of soil microorganisms through a process called **mineralization**. Mineralization depends on many factors, including temperature, water and oxygen availability,

and the type and number of microorganisms present in the soil. As a consequence, rates of mineralization are highly variable, and nutrients from organic residues become available to plants over periods that range from days to months to years. This slow rate of mineralization hinders efficient fertilizer use, so farms that rely solely on organic fertilizers may require the addition of substantially more nitrogen or phosphorus.

Chapter 3. Production of primary and secondary metabolites

1. The light reactions of the photosynthesis

Life on Earth depends on energy derived from the sun. Photosynthesis is the only process of biological importance that can harvest this energy. A large fraction of the planet's energy resources results from photosynthetic activity in either recent or ancient times (fossil fuels). The term photosynthesis means literally "synthesis using light". Photosynthetic organisms use solar energy to synthesize complex carbon compounds. The most active photosynthetic tissue in higher plants is the mesophyll of leaves. Mesophyll cells have many chloroplasts. In the chloroplasts, light energy is converted into chemical energy by two different functional units called *photoystems*. The absorbed light energy is used to power the transfer of electrons through a series of compounds to act as electron donors and electron acceptors. The majority of electrons ultimately reduce NADP+ to NADPH and oxidize H2O to O2. Light energy is also used to generate a proton motive force (PMF) across the thylakoid membrane. This PMF is used to synthesize ATP.

General concept of photosynthesis

Light has both particle and wave characteristics

Light has properties of both particles and waves (**Figure 2.1**). A wave is characterized by a wavelength. The light wave is a transverse (side-to-side) electromagnetic wave, in which both electric and magnetic fields oscillate perpendicularly to the direction of propagation of the wave and at 90o with respect to each other. Sunlight is like a rain of photons of different frequencies. Human eyes are sensitive to only a small range of frequencies – the visible-light region of the electromagnetic spectrum (**Figure 2.2**). The **absorption spectrum** of chlorophyll-a indicates approximately the portion of the solar output that is utilized by plants. An absorption spectrum provides information about the amount of light energy taken up or absorbed by a molecule or substance as a function of the wavelength of the light.

Chlorophyll appears green to our eyes because it absorbs light mainly in the red and blue parts of the spectrum, so only some of the light enriched in green wavelength is reflected into our eyes. Chlorophyll (Chl) in its lowest-energy, or ground state absorbs a photon and makes a transition to a higher-energy, or excited state (Chl*) (**Figure 2.3**). Absorption of blue light excites the chlorophyll to a higher-energy state than absorption of red light, because the energy of photons is higher when their wavelength is shorter. In the higher excited state, chlorophyll is extremely unstable, it very rapidly gives up some of its energy to the surrounding as heat, and enters the lowest excited state, where it can be stable for a maximum of several nanoseconds (10-9 s). Because of the inherent instability of the excited state, any process that captures its energy must be extremely rapid.

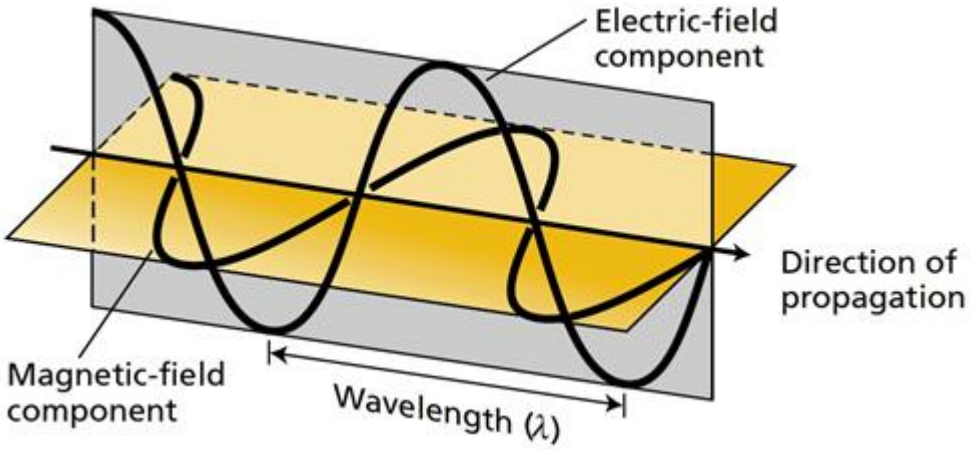


Figure 2.1 Light is a transverse electromagnetic wave, consisting of oscillating electric and magnetic fields (*source: Taiz L., Zeiger E., 2010*)

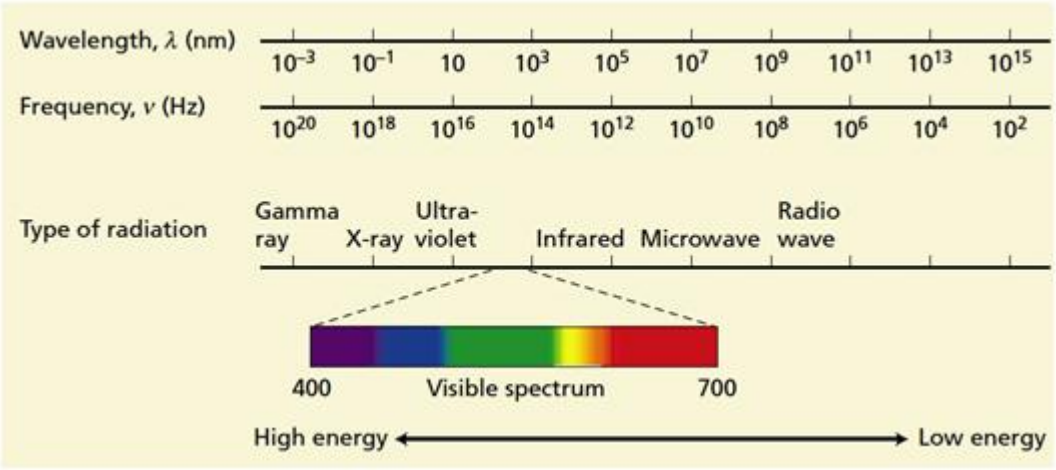


Figure 2.2 The electromagnetic spectrum (*source: Taiz L., Zeiger E., 2010*)

In the lowest excited state, the excited chlorophyll has four alternative pathways for disposing of its available energy:

- Excited chlorophyll can re-emit a photon and thereby return to its ground-state – a process known as fluorescence.
- The excited chlorophyll can return to its ground state by directly converting its excitation energy into heat, with no emission of a photon
- Chlorophyll may participate in energy transfer, during which an excited chlorophyll transfers its energy to another molecule.
- A fourth process is **photochemistry**, in which the energy of the excited state causes chemical reactions to occur. The photochemical reactions of photosynthesis are among the fastest known chemical reactions. This extreme speed is necessary for photochemistry to compete with the three other possible reactions of the excited state.

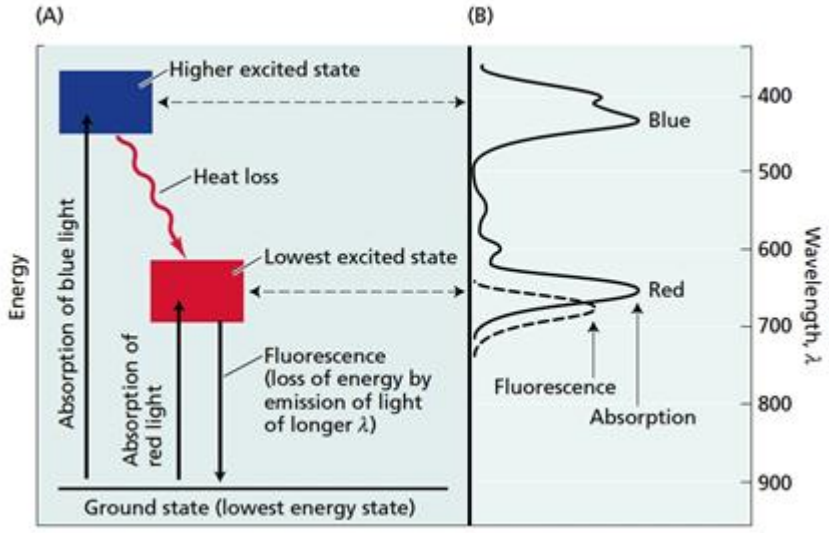


Figure 2.3 Light absorption and emission by chlorophyll (*source: Taiz L., Zeiger E., 2010*)

Photosynthetic pigments absorb the light that powers photosynthesis

The energy of sunlight is first absorbed by the pigments of the plant (**Figure 2.4**). All pigments active in photosynthesis are found in the chloroplast. The **chlorophylls** and bacteriochlorophylls are the typical pigments of photosynthetic organisms. Chlorophylls *a* and *b* are found in green plants, and *c* and *d* are found in some

protists and cyanobacteria. All chlorophylls have a complex ring structure that is chemically related to the porphyrin-like groups found in haemoglobin and cytochromes.

The different type of **carotenoids** found in photosynthetic organisms are all linear molecules with multiple conjugated double bonds. Absorption bands in the 400 to 500 nm region give carotenoids their characteristic orange colour. Carotenoids are found in all photosynthetic organisms. The light energy absorbed by the carotenoids is transferred to chlorophyll for photosynthesis; because of this role they are called accessory pigments. Carotenoids also help to protect the organism from damage caused by light.

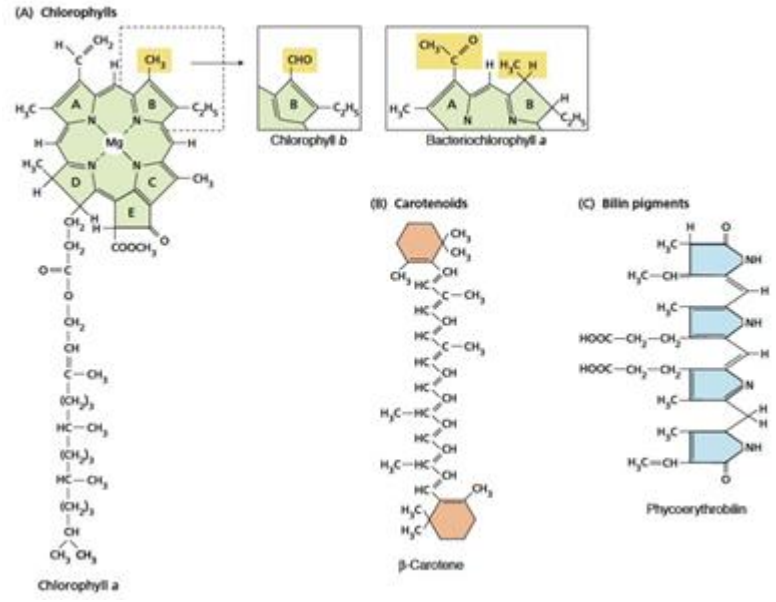


Figure 2.4 Molecular structure of some photosynthetic pigments (*source: Taiz L., Zeiger E., 2010*)

Phycobilisomes serve as the primary light-harvesting antennae for photosystem II in cyanobacteria and red algae. These supramolecular complexes are primarily composed of phycobiliproteins, brilliantly coloured family of water soluble proteins bearing covalently attached, open-chain tetrapyrroles known as **phycobilins**. Absorbed light energy is transferred by very rapid, radiation-less downhill energy transfer from phycoerythrin or phycoerythrocyanin (if present) to C-phycocyanin and then to allophycocyanin species that act as the final energy transmitters from the phycobilisome to the photosystem II or photosystem I reaction centers.

An **action spectrum** depicts the magnitude of a response of a biological system to light as a function of wavelength. For example, an action spectrum for photosynthesis can be constructed from measurements of oxygen evolution at different wavelength. Action spectra were very important for the discovery of two distinct photosystems operating in O2-evolving photosynthetic organisms.

Light-harvesting antennas and photochemical reaction centers

The absorption of the light energy is a cooperation between many chlorophylls and carotenoid molecules (**Figure 2.5**). The majority of the pigments serve as an **antenna complex**, collecting light and transferring the energy to the **reaction center complex**, where the chemical oxidation and reduction reactions leading to long-term energy storage take place. Even in bright sunlight, a single chlorophyll molecule absorbs only a few photons each second. If there were a reaction center associated with each chlorophyll molecule, the reaction center enzymes would be idle most of the time, only occasionally being activated by photon absorption. However, if a reaction center receives energy from many pigments at once, the system is kept active a large fraction of time. Several hundred pigments are associated with each reaction center, and each reaction center must operate four times to produce one molecule of oxygen – hence the value of 2500 chlorophylls per O2. The reaction centers and most of the antenna complexes are integral components of the photosynthetic membrane. In eukaryotic photosynthetic organisms, these membranes are found within the chloroplast; in photosynthetic prokaryotes, the site of photosynthesis is the plasma membrane or membranes derived from it.

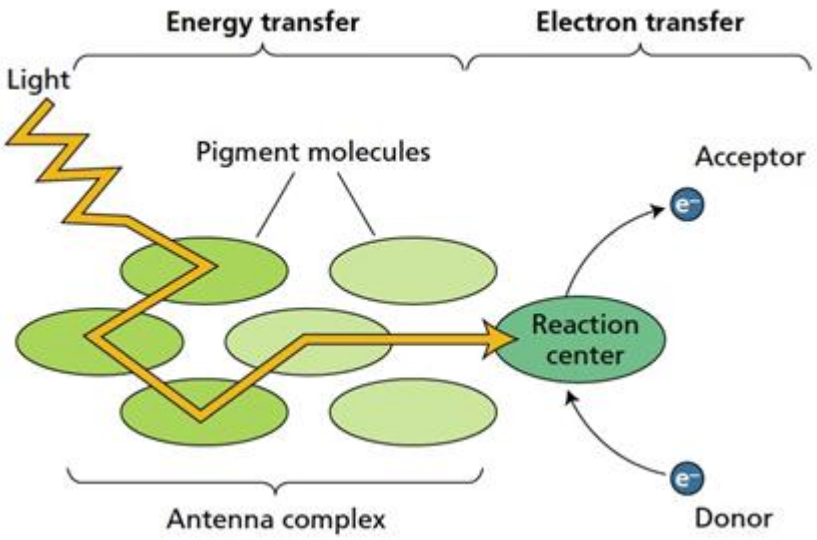


Figure 2.5 Basic concept of energy transfer during photosynthesis (*source: Taiz L., Zeiger E., 2010*)

Oxygen-evolving organisms have two photosystems

The quantum yield of photochemistry is nearly 1.0, the actions of about ten photons are required to produce each molecule of O2, so the overall maximum quantum yield of O2 production is about 0.1. Any photon absorbed by chlorophyll or other pigments is as effective as any other photon in driving photosynthesis. However, the yield drops dramatically in the far-red region of chlorophyll absorption (greater than 680 nm). Emerson discovered the **enhancement effect**. He measured the rate of photosynthesis separately with light of two different wavelength and then used the two beams simultaneously (**Figure 2.6**). When red and far-red light were given together, the rate of photosynthesis was greater than the sum of the individual rates. These and others observations were eventually explained by experiments performed in 1960 that led to the discovery that two photochemical complexes, now known as **photosystem I and II** (**PSI and PSII**), operate in series to carry out the early energy storage reactions of photosynthesis. Photosystem I absorbs far-red light, photosystem II absorbs red light. Another difference between the photosystems is that:

1. Photosystem I produces a strong reductant, capable of reducing NADP+, and a weak oxidant.

2. Photosystem II produces a very strong oxidant, capable of oxidizing water, and a weaker reductant than the one produced by photosystem I.

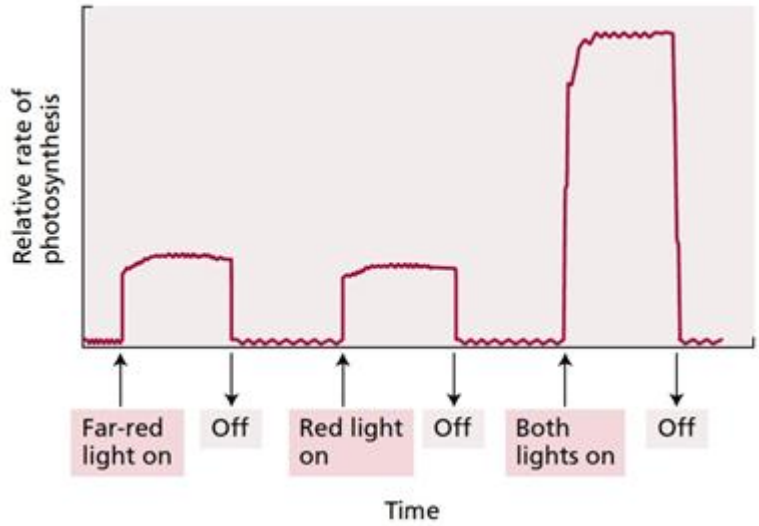


Figure 2.6 The rate of photosynthesis when red and far-red light are given together is greater than the sum of the rates when they are given apart (*source: Taiz L., Zeiger E., 2010*)

Organization of the photosynthetic apparatus

The chloroplast is the site of photosynthesis

In photosynthetic eukaryotes, photosynthesis takes place in the subcellular organelle known as the chloroplast (**Figure 2.7**). The most striking aspect of the structure of the chloroplast is the extensive system of internal membranes known as **thylakoids**, which are the site of the light reactions of photosynthesis. The carbon reduction reactions, which are catalyzed by water-soluble enzymes, take place in the **stroma**, the region of the chloroplast outside the thylakoids. Thylakoid membranes closely associated with each other are known as **grana lamellae**, and the exposed membranes in which stacking is absent are known as **stroma lamellae**. Two separate membranes, each composed of a lipid bilayer and together known as the **envelope**, surround most types of chloroplasts. The chloroplast also contains its own DNA, RNA, and ribosomes.

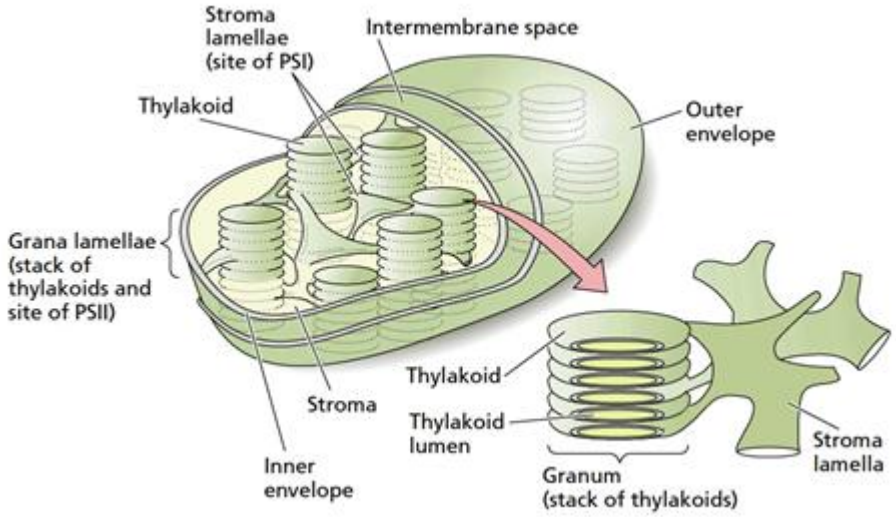


Figure 2.7 Schematic picture of the overall organization of the membranes in the chloroplast (*source: Taiz L., Zeiger E., 2010*)

Thylakoids contain integral membrane proteins

A wide variety of proteins essential to photosynthesis are embedded in the thylakoid membranes. The reaction centers, the antenna pigment-protein complexes, and most of the electron carrier proteins are all integral membrane proteins. Thylakoid membrane proteins have one region pointing toward the stromal side of the membrane and the other oriented toward the interior space of thylakoid, known as **lumen**. The chlorophylls and accessory light-gathering pigments are always pigment-protein complexes. Antenna and reaction center chlorophylls are organized within the membrane so as to optimize energy transfer in antenna complexes and electron transfer in reaction centers.

Photosystem I and II are spatially separated in the thylakoid membrane

The PSII reaction center, along with its antenna chlorophylls and associated electron transport proteins, is located predominantly in the grana lamellae (**Figure 2.8**). The PSI reaction center and its associated antenna pigments and electron transfer proteins, as well as the ATP synthase enzyme that catalyzes the formation of ATP, are found almost exclusively in the stroma lamellae and at the edges of the grana lamellae. The cytochrome b6f complex of the electron transport chain that connects the two photosystems is evenly distributed between stroma and granum lamellae. Thus the two photochemical events that take place in O2-evolving photosynthesis are spatially separated. This separation implies that one or more of the electron carriers that function between the photosystems diffuses from the grana region of the membrane to the stroma region, where electrons are delivered to photosystem I. A strict one-to-one stochiometry between the two photosystems is not required. The ratio of PSII to PSI is about 1.5:1, but it can change when plants are grown in different light conditions.

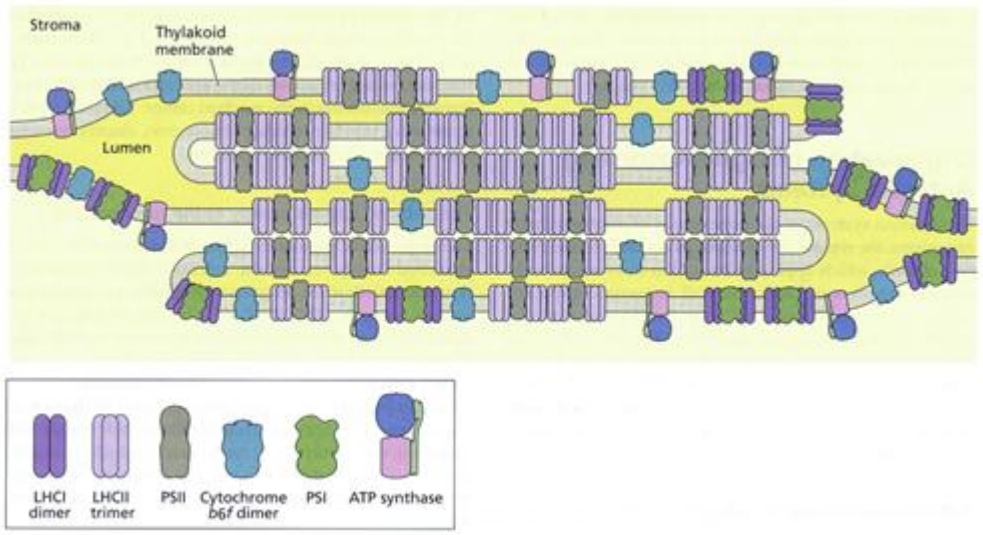


Figure 2.8 Organization of the protein complexes of the thylakoid membrane (*source: Taiz L., Zeiger E., 2010*)

Organization of light-absorbing antenna systems

The antenna systems of different classes of photosynthetic organisms are remarkably varied, in contrast to the reaction centers, which appear to be similar in even distantly related organisms. The variety of antenna complexes reflects evolutionary adaptation to the diverse environments in which different organisms live.

Antenna systems contain chlorophyll and are membrane associated

The size of antenna system varies considerably in different organisms, ranging from 200 to 300 chlorophylls per reaction center in higher plants, to a few thousand pigments per reaction center in some types of algae and bacteria. In almost all cases, the antenna pigments are associated with proteins to form **pigment-protein complexes**. The physical mechanism by which excitation energy is converted from the chlorophyll that absorbs light to the reaction center is **fluorescence resonance energy transfer**, often abbreviated as FRET. By this mechanism the excitation energy is transferred from one molecule to another by a nonradiative process. Approximately 95 to 99% of the photons absorbed by the antenna pigments have their energy transferred to the reaction center, where it can be used for photochemistry. The energy transfer among antenna pigments in the reaction center is a purely physical phenomenon, electron transfer involves chemical (redox) reactions.

The antenna funnels energy to the reaction center

The sequence of pigments within the antenna that funnel absorbed energy toward the reaction center has absorption maxima that are progressively shifted toward longer red wavelength. This red shift in absorption maximum means that energy of the excited state is somewhat lower nearer the reaction center than in the more peripheral portions of the antenna systems. In all eukaryotic photosynthetic organisms that contain both chlorophyll *a* and chlorophyll *b*, the most abundant antenna proteins are members of a large family of structurally related proteins. Some of these proteins are associated primarily with photosystem II and are called **light-harvesting complex II (LHCII)** proteins; others are associated with photosystem I and are called **LHCI** proteins. These antenna complexes are also known as **chlorophyll *a/b* antenna proteins**. The structure of the LHCI proteins is generally similar to that of the LHCII proteins. All of these proteins have significant sequence similarity.

Mechanisms of electron transport

Electrons from chlorophyll travel through the carriers organized in the "Z scheme"

In the "Z scheme" of the O2-evolving photosynthetic organisms all the electron carriers known to function in electron flow from H2O to NADP+ are arranged vertically at their midpoint redox potentials (**Figure 2.9**). Components known to react with each other are connected by arrows, so the Z scheme is really a synthesis of both kinetic and thermodynamic information. The large vertical arrows represent the input of light energy into the system.

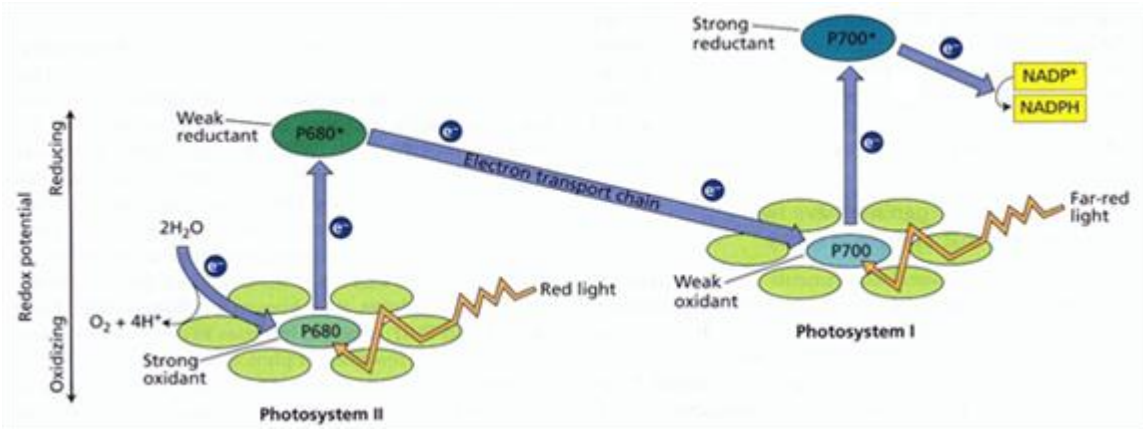


Figure 2.9 "Z scheme" of photosynthesis (*source: Taiz L., Zeiger E., 2010*)

Photons excite the specialized chlorophyll of the reaction centers (P680 for PSII; P700 for PSI), and an electron is ejected. The electron than passes through a series of electron carriers and eventually reduces P700 (for electrons from PSII) or NADP+ (for electrons from PSI). Almost all the chemical processes that make up the light reactions of photosynthesis are carried out by four major protein complexes: photosystem II, the cytochrome b6f complex, photosystem I, and the ATP synthase. These four integral membrane complexes are vectorially oriented in the thylakoid membrane to function as follows (**Figure 2.10**):

1. Photosystem II oxidizes water to O2 in the thylakoid lumen and in the process releases photons into the lumen

2. Cytochrome b6f oxidizes plastohidroquinone (PQH2) molecules that were reduced by PSII and delivers electrons to PSI. The oxidation of plastohidroquinone is coupled to proton transfer into the lumen from the stroma, generating a proton motive force.

3. Photosystem I reduces NADP+ to NADPH in the stroma by the action of ferredoxin (Fd) and the flavoprotein ferredoxin-NADP reductase (FNR).

4. ATP synthase produces ATP as protons diffuse back through it from the lumen into the stroma.

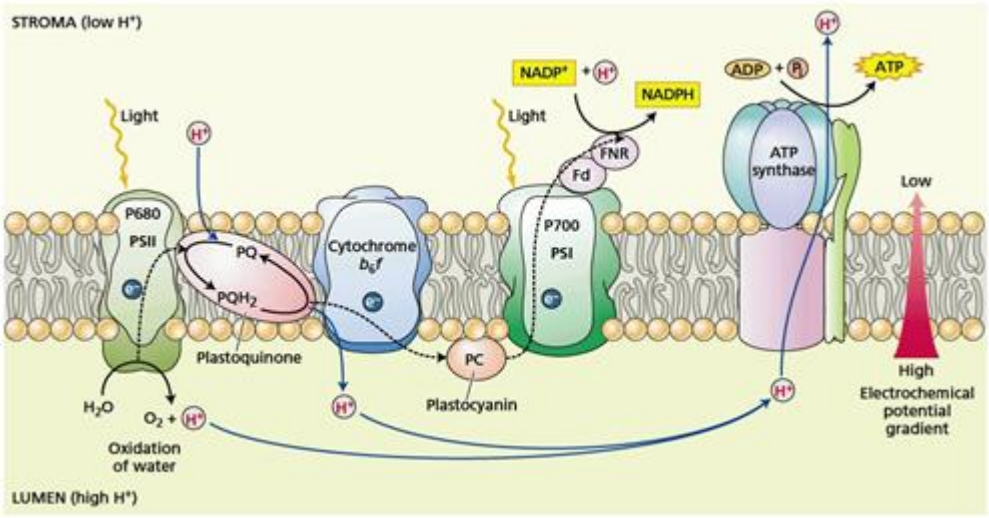


Figure 2.10 The transfer of electrons and protons in the thylakoid membrane is carried out vectorially by four protein complexes (*source: Taiz L., Zeiger E., 2010*)

The photosystem II

PSI and PSII have distinct absorption characteristics. The reaction center chlorophyll of photosystem I absorbs maximally at 700 nm in its reduced state. Accordingly, this chlorophyll is named **P700**. The analogous optical transient of photosystem II is at 680 nm, so its reaction center chlorophyll is known as **P680**.

Photosystem II is contained in a multisubunit protein supercomplex. The core of the reaction center consists of two membrane proteins known as D1 and D2, as well as other proteins. The primary donor chlorophyll, additional chlorophylls, carotenoids, phaeophytins, and plastoquinones are bound to the membrane proteins D1 and D2. Water is oxidized to oxygen by photosystem II. Four electrons are removed from two water molecules, generating an oxygen molecule and four hydrogen ions. The protons are released into the lumen of the thylakoid. These protons are eventually transferred from the lumen to the stroma by translocation through ATP synthase. In this way, the protons released during water oxidation contribute to the electrochemical potential driving ATP formation. Manganese (Mn) is an essential cofactor in the water-oxidizing process. A classic hypothesis in photosynthesis research postulates that Mn ions undergo a series of oxidations – known as S states, and labelled S0, S1, S2, S3, and S4 – that are linked to H2O oxidation and the generation of O2.

Phaeophytin and two quinones accept electrons from photosystem II

In the electron acceptor complex phaephytin acts as an early acceptor in photosystem II. Phaeophytin passes electrons to a complex of two plastoquinones in close proximity to an iron atom. The two plastoquinones, PQA and PQB, are bound to the reaction center and receive electrons from phaeophytin in a sequential fashion. Transfer of the two electrons to PQB reduces it to PQB2-, and the reduced PQB2- takes two protons from the stroma side of the medium, yielding a fully reduced plastohydroquinone (PGH2). The plastohydroquinone transfers its electrons to the cytochrome b6f complex.

Electron flow through the cytochrome b6f complex also transports protons

The **cytochrome b6f complex** is a large multisubunit protein with several prosthetic groups. It is distributed equally between the grana and the stroma regions of the membranes. The precise way by which electrons and protons flow through the cytochrome b6f complex is not yet fully understood, but a mechanism known as the **Q cycle** accounts for most of the observations. In this mechanism, plastohydroquinone (PQH2) is oxidized, and one of the two electrons is passed along a linear electron transport chain toward photosystem I, while the other electron goes through a cyclic process that increases the number of protons pumped across the membrane. In the linear transport chain, the oxidized Rieske protein (**FeSR**) accepts an electron from PQH2 and transfers it to cytochrome *f*. Cytochrome f then transfers an electron to the blue-coloured copper protein plastocyanin (PC), which in turn reduces oxidized P700 of PSI. The **plastocyanin (PC)** is a small, water soluble, copper-containing protein that transfers electrons between the cytochrome b6f complex and P700. This protein is found in the lumenal space.

The photosystem I reaction center reduces NADP+

The PSI reaction center complex is a large multisubunit complex. In contrast to PSII, in which the antenna chlorophylls are associated with the reaction center, but present on separate pigment-proteins, a core antenna consisting of about 100 chlorophylls is an integral part of the PSI reaction center. The core antenna and P700 are bound to two proteins, PsaA and PsaB. Electrons from PSI reaction center are transferred to **ferredoxin (Fd)**, a small, water-soluble iron-sulfur protein. The membrane-associated **flavoprotein ferredoxin-NADP-reductase (FNR)** reduces NADP+ to NADPH, thus completing the sequence of noncyclic electron transport that begins with the oxidation of water.

Some of the cytochrome b6f complexes are found in the stroma region of the membrane, where photosystem I is located. Under certain conditions, **cyclic electron flow** is known to occur from the reducing side of photosystem I via plastohydroquinone and the b6f complex and back to P700. This cyclic electron flow is coupled to proton pumping into the lumen, which can be utilized for ATP synthesis but does not oxidize water or reduce NADP+.

Proton transport and ATP synthesis in the chloroplast

A fraction of the captured light energy is used for light-dependent ATP-synthesis, which is known as photophosphorilation. It is widely accepted that photophosphorilation works via the **chemiosmotic mechanism**, which was first proposed in the 1960s by Peter Mitchell. Chemiosmosis appears to be a unifying aspect of membrane processes in all forms of life. The basic principle of chemiosmosis is that ion concentration differences and electric-potential differences across membranes are sources of free energy that can be utilized by the cell. Electron flow is accompanied with the proton flow from one side of the membrane to the other. The direction of proton translocation is such that the stroma becomes more alkaline (fewer H+ ions) and the lumen becomes more acidic (more H+ ions) as a results of electron transport. Mitchell proposed that the total energy available for ATP synthesis, which he called the **proton motive force**, is the sum of a proton chemical potential

and a transmembrane electric potential. Transmembrane pH difference of one pH unit is equivalent to a membrane potential of 59 mV.

The ATP is synthesized by an enzyme complex known by several names: **ATP synthase, ATPase, and CF0-CF1**. This enzyme consists of two parts: a hydrophobic membrane-bound portion called CF0 and a portion that sticks out into the stroma called CF1. Remarkable aspect of the mechanism of he ATP synthase is that the internal stalk and probable much of the CF0 of the enzyme rotate during catalysis. The enzyme is actually a tiny molecular motor. Three molecules of ATP are synthesized for each rotation of the enzyme. The stoichiometry of protons translocated to ATP formed is 14/3, or 4.67.

2. Carbon reactions of the photosynthesis

Solar radiant energy (ca. 3 x 1021 Joules/year) is converted via endergonic reactions in plants into carbohydrates (ca. 2 x 1011 tonnes of carbon/year). The capture of sunlight energy for transformation into various forms of chemical energy is one of the oldest biochemical reactions on Earth. One billion years ago, heterotrophic cells acquired the ability to convert sunlight into chemical energy through primary endosymbiosis with a cyanobacterium. The original endosymbiosis has given rise to an enormous variety of organelles. In general, the transition from endosymbiont to organelle involved both the loss of functions unnecessary in the protected milieu of the host cell and the gain of other metabolic pathways. The chloroplast is the place of both the light and carbon reactions of photosynthesis.

The products of the light reactions, ATP and NADPH, flow from thylakoid membranes to the surrounding fluid phase (stroma) and drive the enzyme-catalyzed reduction of atmospheric CO2 to carbohydrates and other cell components. Because the stroma-localized reactions depend on products of the photochemical processes and are also known to be regulated directly by light, they are more properly referred to as *carbon reactions of photosynthesis*. The incorporation of atmospheric CO2 into organic compounds appropriate for life is accomplished by the **Calvin-Benson cycle**. There are two major products of the photosynthetic fixation of CO2: starch, the reserve polysaccharide that accumulates transiently in chloroplasts; and sucrose, the disaccharide that is exported from leaves to developing and storage organs of the plant.

The Calvin-Benson cycle

The Calvin-Benson cycle is found in many prokaryotes and in all photosynthetic eukaryotes, from the most primitive algae to the most advanced angiosperms. It is also aptly named the *reductive pentose phosphate cycle*.

The Calvin-Benson cycle has three stages

The Calvin-Benson cycle was elucidated by M. Calvin, A. Benson and their colleagues in the 1950s. It proceeds in three stages that are highly coordinated in the chloroplast (**Figure 2.11**):

1. *Carboxylation* of the CO2 acceptor molecule. The first committed enzymatic step to generate two molecules of a 3-carbon intermediate (3-phosphoglycerate).

2. *Reduction* of 3-phosphoglycerate.

3. *Regeneration* of the CO2 acceptor ribulose 1,5-bisphosphate.

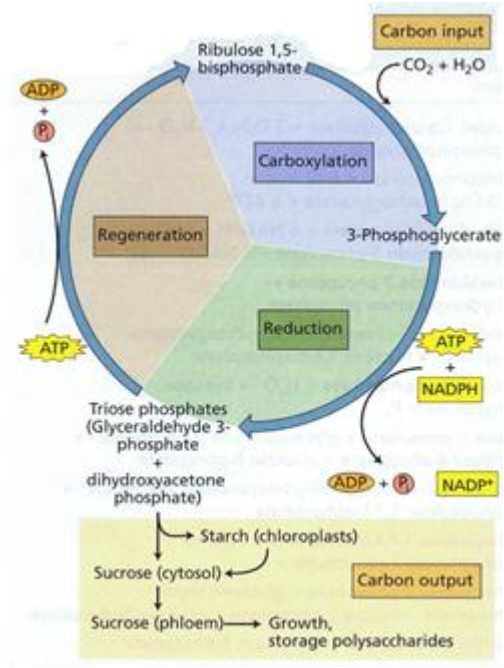


Figure 2.11 The Calvin-Benson cycle proceeds in three stages: carboxylation, reduction, and regeneration (*source: Taiz L., Zeiger E., 2010*)

In the first step three molecules of CO2 and three molecules of H2O react with three molecules of ribulose 1,5-bisphosphate to yield six molecules of 3-phosphoglycerate. This reaction is catalyzed by the chloroplast enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase, referred to as **rubisco**. The reduction stage of the Calvin-Benson cycle reduces the carbon of the 3-phosphoglycerate coming from the carboxylation stage. To prevent depletion of Calvin-Benson cycle intermediates, the continuous uptake of atmospheric CO2 requires constant regeneration of the CO2 acceptor ribulose 1,5-bisphosphate.

Triose phosphates are formed in the carboxylation and reduction phases of the Calvin-Benson cycle at the expense of energy (ATP) and reducing equivalents (NADPH) generated in the thylakoid membranes of chloroplasts:

3CO2 + 3 ribulose 1,5-bisphosphate + 3H2O + 6NADPH + 6H+ +6ATP ---> 6 triose phosphates + 6NADP+ + 6ADP +6Pi

From these six triose phosphates, five are used in the regeneration phase that restores ribulose 1,5-bisphosphate, the CO2 acceptor, while the sixth triose phosphate represents net synthesis from CO2 and is used as a building block for other metabolic processes.

5 triose phosphates + 3ATP ---> 3 ribulose 1,5-bisphosphate + 3ADP

In summary, the fixation of three CO2 into one triose phosphate utilizes 9ATP and 6NADPH; that is, the ratio of ATP:NADPH required for the fixation of one CO2 in the Calvin-Benson cycle is 3:2.

When leaves are kept in darkness for long periods (e.g., at night), the stromal concentration of most biochemical intermediates of the Calvin-Benson cycle is low. Therefore, when leaves are transferred to the light, almost all stromal triose phosphates are committed to the production of the intermediates necessary to regenerate ribulose 1,5-bisphosphate. The fixation of CO2 starts after a lag, called the *induction period*, and the rate of photosynthesis increases with time in the first few minutes after the onset of illumination.

Regulation of the Calvin-Benson cycle

The efficient use of energy in the Calvin-Benson cycle requires the existence of specific regulatory mechanisms ensuring not only that all intermediates in the cycle are present at adequate concentrations in the light, but also that the cycle is turned off when not needed in the dark. Although rubisco plays a critical role in the carbon cycle of the biosphere, its catalytic rate is extremely slow (1-12 CO2 fixations per second). This paradoxical feature was clarified when George Lorimer and colleagues found that rubisco must be activated before acting as

a catalyst. Further studies revealed that the CO2 molecule plays a dual role in the activity of rubisco: CO2 participates in the transformation of the enzyme from an inactive to an active form (modulation) and is the substrate for the carboxylase reaction (catalysis).

In addition to rubisco, light controls the activity of four other enzymes of the Calvin-Benson cycle via the ferredoxin-thioredoxin system, which consists of ferredoxin, ferredoxin-thioredoxin reductase, and thioredoxin. The deactivation of the target enzymes in the dark appears to take place by reversal of the reduction (activation) pathway. Oxygen or reactive oxygen species transform reduced thioredoxin (-SH HS-) to the oxidized state (-S-S-), which in turn converts the reduced target enzyme to the oxidized state, leading to loss of catalytic activity.

Upon illumination, the flow of protons from the stroma into the thylakoid lumen is coupled to the release of Mg2+ from the intrathylakoid space to the stroma. These ion fluxes decrease the stromal concentration of H+ (the pH increases from 7 to 8) and increase that of Mg2+ by 2-5mM. Several Calvin-Benson cycle enzymes that require Mg2+ for catalysis are more active at pH 8 than at pH 7, including rubisco, fructose-1,6-bisphosphatase, sedoheptulose-1,7-bisphosphatase, and phosphoribulokinase. Hence, the light-mediated increase of Mg2+ and H+ enhances the activity of key enzymes of the Calvin-Benson cycle.

The C2 oxydative photosynthetic carbon cycle

Rubisco has the capacity to catalyze both the carboxylation and oxygenation of ribulose 1,5-bisphosphate. Carboxylation yields two molecules of 3-phosphoglycerate, while oxygenation produces one molecule each of 3-phosphoglycerate and 2-phosphoglycolate. The oxygenation of ribulose 1,5-bisphosphate catalyzed by rubisco initiates a coordinated network of enzymatic reactions that are compartmentalized in chloroplasts, leaf peroxisomes, and mitochondria (**Figure 2.12**). This process, known as **photorespiration**, causes the partial loss of CO2 fixed by the Calvin-Benson cycle. That is why several crops show a dramatic increase in yield when grown in greenhouse with elevated levels of CO2.

Carboxylation and oxygenation are competing reactions

The ability to catalyze the oxygenation of ribulose 1,5-bisphosphate is a property of all rubiscos, regardless of taxonomic origin. The 2-phosphoglycolate formed in the chloroplast by oxygenation of ribulose 1,5-bisphosphate is rapidly hydrolyzed to glycolate by a specific chloroplast phosphatase. The subsequent metabolism of glycolate involves the cooperation of two other organelles: peroxisomes and mitochondria. Glycolate exits the chloroplast via a specific transporter protein and diffuses to the peroxisome. The glycolate oxidase catalyzes the oxidation of glycolate by producing H2O2 and glyoxylate. Catalase breaks down the H2O2, releasing O2, while glyoxylate undergoes transamination with glutamate, yielding the amino acid glycine. Glycine leaves the peroxisome and enters the mitochondrion, where two molecules of glycine are converted to serine and CO2. The newly formed serine diffuses from the mitochondrion back to the peroxisome, where it is converted to glycerate. Finally, glycerate reenters the chloroplast, where it is phosphorilated to yield 3-phosphoglycerate.

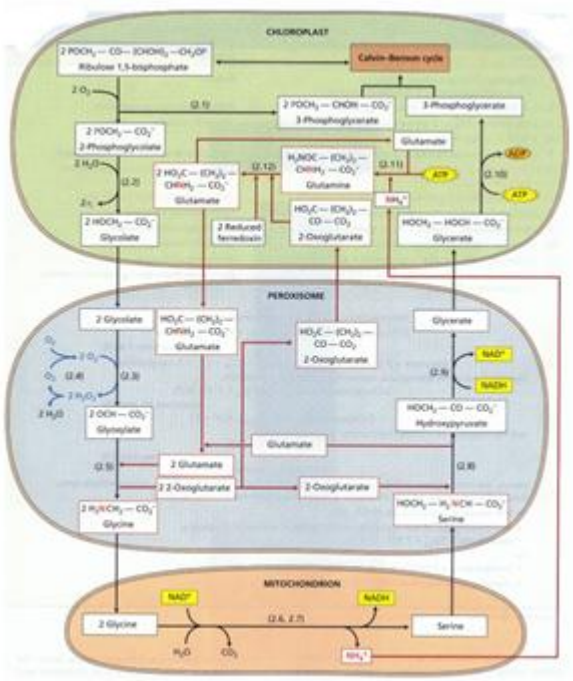


Figure 2.12 Operation of the C2 oxidative photosynthetic cycle involves the cooperative interaction among three organelles (*source: Taiz L., Zeiger E., 2010*)

In vivo, the balance between the Calvin-Benson and the C2 oxidative photosynthetic cycles is determined mainly by three factors: one is inherent to the plant (the kinetic properties of rubisco), and two are linked to the environment (temperature and the concentration of substrates, CO2 and O2). An increase in the external temperature:

- modifies the kinetic constants of rubisco, increasing the rate of oxygenation more than that of carboxylation, and
- lowers the concentration of CO2 more than that of O2 in a solution in equilibrium with air.

Hence, the increase in photorespiration (oxygenation) relative to photosynthesis (carboxylation) significantly limits the efficiency of photosynthetic carbon assimilation under warmer temperatures. Overall, a progressive increase in temperature tilts the balance away from the Calvin-Benson cycle and toward the C2 oxidative photosynthetic cycle.

Inorganic carbon-concentrating mechanisms

The pronounced reduction in CO2 and rise in O2 levels that commenced about 350 million years ago triggered a series of adaptations to handle an environment that promoted photorespiration in photosynthetic organisms. These adaptations include various strategies for active uptake of CO2 and HCO3- from the surrounding environment and subsequent accumulation of inorganic carbon near rubisco.

The C4 cycle

To minimize the oxygenase activity of rubisco and the concurrent loss of carbon through the photorespiratory cycle, C4 photosynthesis appears to have evolved as one of the major carbon-concentrating mechanisms used by land plants to compensate for limitations associated with the low level of atmospheric CO2. M.D. Hatch and C.R. Slack elucidated what is now named the C4 photosynthetic carbon cycle (also known as the Hatch-Slack cycle or the C4 cycle). They established that malate and aspartate are the first stable, detectable intermediates of photosynthesis in leaves of sugarcane. This novel metabolic pathway takes place in two morphologically distinct cell types, the mesophyll and bundle sheet cells. In the C4 cycle, the enzyme phosphoenolpyruvate carboxylase (PEPCase), rather than rubisco, catalyzes the primary carboxylation in a tissue that is close to the external atmosphere. The resulting 4-carbon acid flows across the diffusion barrier to the vascular region, where it is decarboxylated, releasing CO2 that is refixed by rubisco via the Calvin-Benson cycle.

Two different types of cells participate in the C4 cycle

The key features of the C4 cycle were initially found in leaves of plants whose vascular tissues are surrounded by two distinctive photosynthetic cell types, an internal ring of **bundle sheath cells**, which is wrapped with an outer ring of **mesophyll cells**. The chloroplasts in bundle sheath cells are concentrically arranged and exhibit large starch granules and unstacked thylakoid membranes. On the other hand, mesophyll cells contain randomly arranged chloroplasts with stacked thylakoids and little or no starch. In this anatomical context, the transport of CO2 from the external atmosphere to the bundle sheath cells proceeds through five successive stages (**Figure 2.13**):

1. fixation of the HCO3- by PEPCase in the mesophyll cells,

2. transport of the 4-carbon acids (malate, aspartate) to bundle sheath cells,

3. decarboxylation of the 4-carbon acids and generation of CO2, which is then reduced to carbohydrate via the Calvin-Benson cycle,

4. transport of the 3-carbon backbone (pyruvate or alanine) back to the mesophyll cells,

5. regeneration of the HCO3- acceptor.

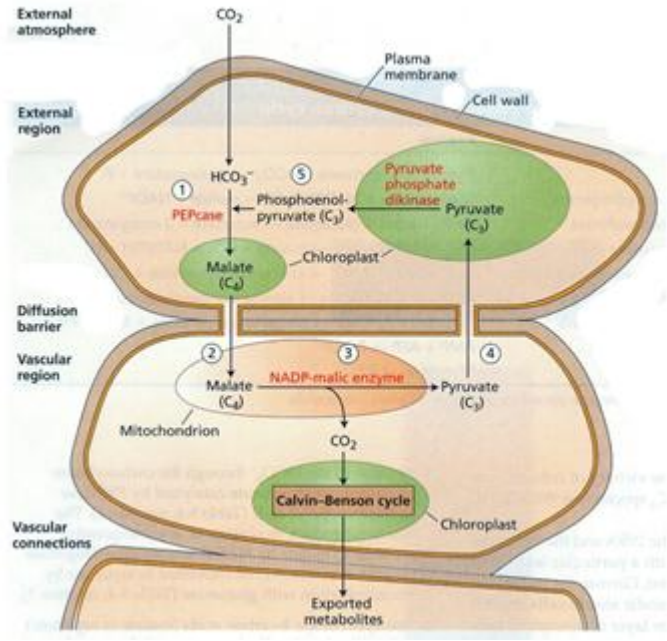


Figure 2.13 The C4 photosynthetic carbon cycle (*source: Taiz L., Zeiger E., 2010*)

The compartmentalization of enzymes ensures that inorganic carbon from the surrounding atmosphere can be taken up initially by mesophyll cells, fixed subsequently by the Calvin-Benson cycle of bundle sheath cells, and finally exported to the phloem.

The C4 cycle is known to occur in 18 families of both monocots and dicots. In all cases the operation of the C4 cycle requires the cooperative effort of the two distinct chloroplast-containing cell types. The transport process facilitated by plasmodesmata connecting the two cell types generates a much higher concentration of CO2 in bundle sheath cells (the vascular region) than in mesophyll cells. The elevated concentration of CO2 at the carboxylation site of rubisco results in the suppression of ribulose 1,5-bisphosphate oxygenation and hence of photorespiration.

Chloroplasts from mesophyll cells of C3 and C4 plants exhibit qualitatively similar but quantitatively different proteomes in their envelope membranes. In particular, translocators that participate in the transport of triose phosphates and phosphoenolpyruvate are more abundant in the envelopes of C4 plants than in those of C3 plants. This higher abundance ensures that fluxes of metabolic intermediates across the chloroplast envelope in C4 plants are higher than in C3 plants.

The C4 cycle reduces photorespiration and water loss

Elevated temperature decrease both the carboxylative capacity of rubisco and the solubility of CO2, thus limiting the rate of photosynthetic CO2 assimilation in C3 plants. In C4 plants, two features overcome the deleterious effects of high temperature:

- first, the affinity of PEPCase for its substrate, HCO3-, is sufficiently high to saturate the enzyme at the reduced CO2 levels present in warm climates. Further, oxygenase activity is largely suppressed because HCO3- does not compete with O2 in the initial carboxylation. This high activity of PEPCase enables C4 plants to reduce their tomatal aperture at high temperatures and thereby conserve water while fixing CO2 at rates equal to or greater that those of C3 plants.

- second, the high concentration of CO2 in bundle sheath cells minimizes the operation of the C2 oxidative photosynthetic cycle.

Crassulacean acid metabolism (CAM)

Many plants that inhabit arid environments with seasonal water availability, including commercially important plants, such as pinapple, agave, cacti, and orchids, exhibit another mechanism for concentrating CO2 at the site of rubisco. This important variant of photosynthetic carbon fixation was historically named crassulacean acid metabolism (CAM) to recognize its initial observation in *Bryophyllum calycinum*, a succulent member of the Crassulaceae. An important attribute of CAM plants is their capacity to attain high biomass in habitats where precipitation is inadequate, or where evaporation is so great that rainfall is insufficient for crop growth. CAM is generally associated with anatomical features that minimize water loss, such as thick cuticles, low surface-to-volume ratios, large vacuoles, and stomata with small apertures. In addition, tight packing of the mesophyll cells enhances CAM performance by restricting CO2 loss during the day.

In CAM plants, the initial capture of atmospheric CO2 into C4 acids and the final incorporation of CO2 into carbon skeletons are spatially close but temporally out of phase – by almost 12 hours over the 24-hour light-dark cycle (**Figure 2.14**). At night, cytosolic PEPCase fixes atmospheric (and respiratory) CO2 into oxaloacetate using phosphoenolpyruvate formed via the glycolytic breakdown of stored carbohydrates. A cytosolic NAD-malate dehydrogenase converts the oxaloacetate to malate, which is stored in the acid vacuole for the remainder of the night. During the day, the stored malate is transported to the chloroplast and decarboxylated. The released CO2 is made available to the chloroplast for processing via the Calvin-Benson cycle, while the complementary 3-carbon acids are converted to triose phosphates and subsequently to starch or sucrose via gluconeogenesis as in C4 plants.

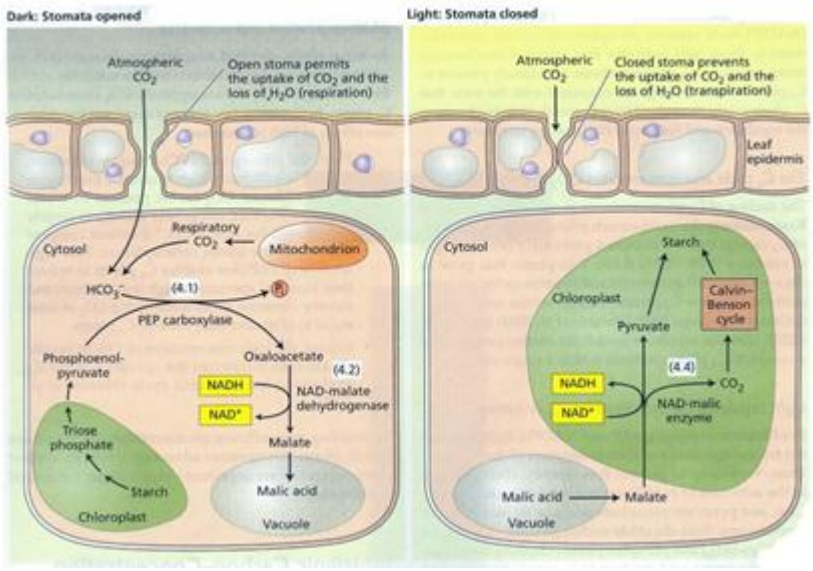


Figure 2.14 Inorganic carbon-concentrating mechanism: crassulacean acid metabolism (CAM) (*source: Taiz L., Zeiger E., 2010*)

Changes in the rate of carbon uptake and in enzyme regulation throughout the day create a 24-hour CAM cycle that is divided into four distinct phases: phase I (night), phase II (early morning), phase III (daytime), and phase

IV (late afternoon). During the nocturnal phase I, when stomata are open and leaves are respiring, CO2 is captured and stored as malate in the vacuole. CO2 uptake by PEPCase dominates phase I. In the diurnal phase III, when stomata are closed and leaves are photosynthesizing, the stored malate is decarboxylated. This results in high concentrations of CO2 around the active site of rubisco, thereby alleviating the adverse effects of photorespiration. The transient phases II and IV shift the metabolism in preparation for phases III and I, respectively. In phase II, rubisco activity increases, but it decreases in phase IV. In contrast the activity of PEPCase increases in phase IV, but declines in phase II.

CAM is a versatile mechanism sensitive to environmental stimuli

CAM plants that grow in deserts, such as cacti, open their stomata during the cool nights and close them during the hot, dry days. Closing the stomata during the day minimizes the loss of water but, because H2O and CO2 share the same diffusion pathway, CO2 must then be taken up by the open stomata at night. When the stomata are closed, neither the CO2 released by decarboxylating enzymes nor the CO2 released in mitochondrial respiration escape from the leaf. As a consequence, the internally generated CO2 is fixed and converted to carbohydrates by the Calvin-Benson cycle. Thus, stomatal closure not only helps conserve water, but also assists in the building of the elevated internal concentration of CO2, that enhances the photosynthetic carboxylation of ribulose 1,5-bisphosphate.

The water-conserving closure of stomata in arid lands may not be the unique basis of CAM evolution, because, paradoxically, CAM species are also found among aquatic plants. Perhaps this mechanism also enhances the acquisition of inorganic carbon (as HCO3-) in aquatic habitats, where high resistance to gas diffusion restricts the availability of CO2.

Accumulation and partitioning of photosynthates – starch and sucrose

Eukaryotic organisms have to mobilize sugars from the site of synthesis or absorption (source) to cells that use them for growth or energy (sinks). The photosynthetic assimilation of CO2 by most leaves yields sucrose and starch as end products, but the pathways that produce them are physically separated: sucrose is synthesized in the cytosol and starch in chloroplasts (**Figure 2.15**). During the day, sucrose flows continuously from the leaf cytosol to heterotrophic sink tissues, while starch accumulates as dense granules in chloroplasts. Sucrose is the principal carbohydrate exported from source leaves to sink tissues in most plants. The retention of some photosynthate as starch in the chloroplast during the day ensures that there will be carbohydrate available for conversion to sucrose for export at night. Plants vary widely in the extent to which they accumulate starch and sucrose in leaves.

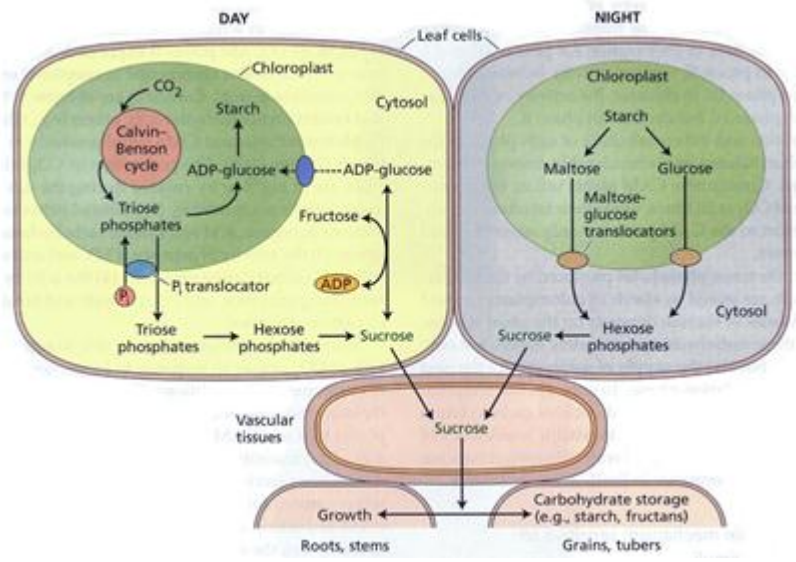


Figure 2.15 Accumulation and partitioning of photosynthates: starch and sucrose (*source: Taiz L., Zeiger E., 2010*)

Environmental factors also influence the amount of fixed carbon allocated to sucrose and starch in the leaf; plants grown in short days divert relatively more of their photosynthates to starch than their counterparts grown in long days, thus ensuring an adequate supply of sugars during the longer nights. Sugars produced by

photosynthesis are transported from the source (leaf cells) to nonphotosynthetic sinks (stems, roots, tubers, grains) through the vascular tissues (phloem).

The onset of darkness not only stops the assimilation of CO2, but also starts the degradation of chloroplast starch. The content of starch in the chloroplast falls dramatically through the night, as it is converted to sucrose and exported. Low levels of sugars in sink tissues stimulate the rate of photosynthesis and the mobilization of carbohydrates from reserve organs. On the other hand, an abundance of sugars in leaves promotes plant growth and carbohydrate storage in reserve organs.

3. Photosynthetic activity and environmental factors

Several environmental factors influence the photosynthesis, which shows direct responses to the environmental factors like light, ambient CO2 concentrations, and temperature, as well as indirect responses (mediated through the effects of stomatal control) like humidity and soil moisture. However, under any particular conditions, the rate of photosynthesis is limited by the slowest step in the process, the so-called *limiting factor*. Therefore, at any given time, photosynthesis can be limited either by light or by CO2 concentration, but not by both factors at the same time.

Photosynthesis is the primary function of leaves

Leaves are exposed to different spectra and quantities of light that result in photosynthesis. The light reaching the plant is a flux and that flux can be measured in either energy or photon units. Irradiance (energy) is expressed in watts per square meter (W m-2; 1 W = 1 joule s-1). Photon irradiance is the number of incident quanta, expressed in moles per square meter per second (mol m-2 s-1; 1 mol of light = 6.02 x 1023 photons). The **photosynthetically active radiation** (**PAR**, 400-700nm) may also be expressed in terms of energy (W m-2) but is more commonly expressed as quanta (mol m-2 s-1). Under direct sunlight, PAR irradiance is about 2000 µmol m-2 s-1 (900 W m-2) at the top of a dense forest canopy, but may be only 10 µmol m-2 s-1 (4.5 W m-2) at the bottom of the canopy. While roughly 1.3 kW m-2 of radiant energy from the sun reaches Earth, less than 5% of this energy is ultimately converted into carbohydrates by a photosynthesizing leaf. Significant fraction of the absorbed light is lost as heat and a smaller amount is lost as fluorescence.

Leaf anatomy maximizes light absorption

The anatomy of the leaf is highly specialized for light absorption. The epidermis is typically transparent to visible light. Below the epidermis, the top layers of photosynthetic cells are called palisade cells. Some leaves have several layers of columnar palisade cells. To increase the efficiency of photosynthetic structures within palisade cells, chloroplasts have high surface-to-volume ratios. Below the palisade layers is the spongy mesophyll, where the cells are very irregular in shape and are surrounded by large air spaces. The large air spaces generate many interfaces between air and water that reflect and refract the light, thereby randomizing its direction of travel. This phenomenon is called **interface light scattering**. Some environments, such as deserts, have so much light that it is potentially harmful to leaves. In these environments leaves often have special anatomical features, such as hairs, salt glands, and epicuticular wax, that increase the reflection of light from the leaf surface, thereby reducing light absorption by as much as 40%.

Leaf angle and leaf movement can control light absorption

Under natural conditions, leaves exposed to full sunlight at the top of canopy tend to have steep leaf angles, which allow more sunlight to penetrate into the canopy. It is common to see the angle of leaves within a canopy decrease (become more horizontal) with increasing depth in the canopy. Some plants control light absorption by **solar tracking**, that is, their leaves continuously adjust the orientation of their laminae such that they remain perpendicular to the sun's rays. Many species, including alfalfa, cotton, soybean, bean, and lupine, have leaves capable of solar tracking (**Figure 2.16**). Solar tracking is a blue-light response.

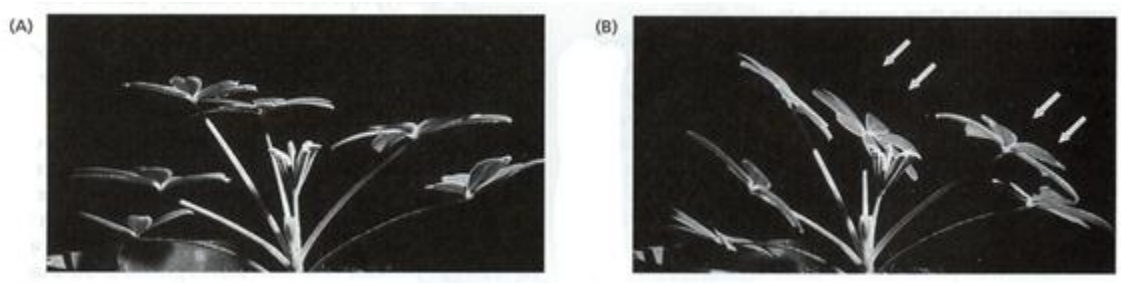


Figure 2.16 Leaf movement in sun-tracking plants: (A) initial orientation, and (B) orientation 4 hours after exposure to light (*source: Taiz L., Zeiger E., 2010*)

The term heliotropism used to describe sun-induced leaf movements, we call leaves that maximize light interception by solar tracking *diaheliotropic*. Some solar tracking plants can also move their leaves so that they avoid full exposure to sunlight, thus minimizing heating and water loss. These sun avoiding leaves are called *paraheliotropic*. Some plant species have leaves that can display diaheliotropic movements when they are well watered and paraheliptropic movements when they experience water stress. Diaheliotropic solar tracking appears to be a feature common to wild plants that are short-lived and must complete their life cycle before the onset of drought. Paraheliotropic leaves are able to regulate the amount of sunlight incident on the leaf to a nearly constant value. Often only one-half to two-thirds of full sunlight may be advantageous under conditions of water stress or excessive solar radiation.

Plants acclimate and adapt to sun and shade environments

Acclimation is a growth process in which each newly produced leaf has a set of biochemical and morphological characteristics suited to the particular environment in which it unfolds. In some plant species the mature leaf will abscise and a new leaf will develop that is better suited for the new environment. However, some species of plants are not able to acclimate when transferred from a sunny to a shady environment. These plants are **adapted** to either a sunny or a shady environment. When plants adapted to deep shade conditions are transferred into full sunlight, the leaves experience chronic photoinhibition and leaf bleaching, and the plants eventually die. Shade leaves have more total chlorophyll per reaction centre, have a higher ratio of chlorophyll *b* to chlorophyll *a*, and are usually thinner that sun leaves. Sun leaves have more rubisco, are thicker, and have longer palisade cells than leaves grown in the shade. The adaptive response of some shade plants is to produce a 3:1 ratio of photosystem II to photosystem I reaction centers, compared with the 2:1 ratio found in sun plants. Other shade plants add more antenna chlorophyll to PSII to increase absorption by this photosystem and better balance to flow of energy through PSII and PSI. These changes appear to enhance light absorption and energy transfer in shady environment.

Photosynthetic responses to light by the intact leaf

In the dark CO2 is given off by the plant because of mitochondrial respiration. With increasing irradiance photosynthetic CO2 assimilation eventually reaches a point at which photosynthetic CO2 uptake exactly balances the respiratory CO2 release. This is called the **light compensation point**. Light compensation points of sun plants range from 10 to 20 µmol m-2 s-1, whereas corresponding values for shade plants are 1 to 5 µmol m-2 s-1. The linear relationship between photon flux and photosynthetic rate persists at light levels above the light compensation point. The slope of this linear portion of the curve reveals the **maximum quantum yield** of photosynthesis for the leaf. Leaves of sun and shade plants show very similar quantum yields. This is because the basic biochemical processes that determine quantum yield are the same for these two types of plants. The quantum yield of photochemistry is about 0.95. However, the photosynthetic quantum yield is lower (0.125 for C3 plants). The quantum yields for CO2 of C3 and C4 leaves vary between 0.04 and 0.06 mole of CO2 per mole of photons. If C3 leaves are exposed to low O2 concentrations, photorespiration is minimized and the quantum yield increases to about 0.09 mole of CO2 per mole of photons. At higher photon fluxes, the photosynthetic response to light starts to level off and eventually reaches saturation. The light-response curve of most leaves saturates between 500 and 1000 µmol m-2 s-1, well below full sunlight. However, because the photosynthetic response of the intact plant is the sum of the photosynthetic activity of all the leaves, only rarely is photosynthesis light-saturated at the level of the whole plant.

Leaves must dissipate excess light energy

When exposed to excess light, leaves must dissipate the surplus absorbed light energy so that it does not harm the photosynthetic apparatus. Heat production and the xanthophylls cycle appears to be important avenues for dissipation of excess light energy. The xanthophylls cycle comprises the three carotenoids violaxanthin, antheraxanthin, and zeaxanthin. Experiments have shown that zeaxanthin is the most effective of the three xanthophylls in heat dissipation. The zeaxanthin content increases at high irradiances and decreases at low irradiances. In leaves growing under full sunlight, zeaxanthin and antheraxanthin can make up 60% of the total xanthophyll cycle pool at maximal irradiance levels attained at midday (**Figure 2.17**). Contrary to the diurnal cycling of this pool observed in summer, zeaxanthin levels remain high all day during the winter. Presumably this mechanism maximizes dissipation of light energy, thereby protecting the leaves against photooxidation during winter.

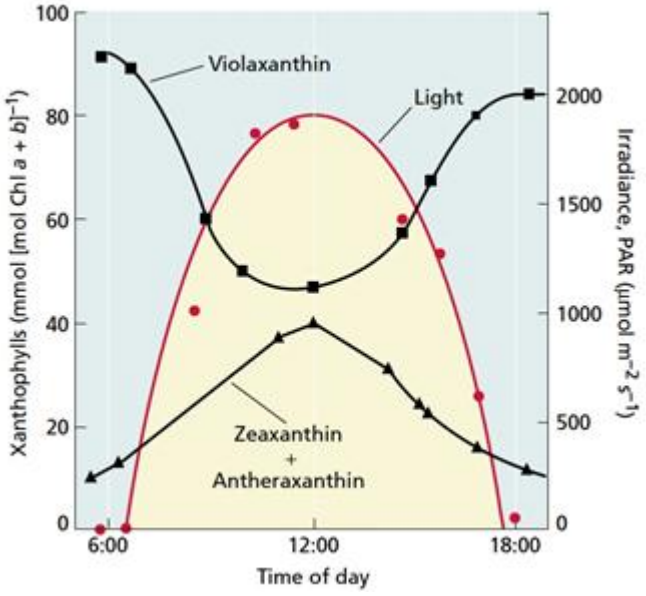


Figure 2.17 Diurnal changes in xanthophyll content as a function of irradiance in sunflower (*source: Taiz L., Zeiger E., 2010*)

An alternative means of reducing excess light energy is to move the chloroplasts so that they are no longer exposed to high light. Under high light, the chloroplasts move to the cell surfaces that are parallel to the incident light, thus avoiding excess absorption of light. Such chloroplast rearrangement can decrease the amount of light by the leaf about 15%. Chloroplast movement in leaves is a typical blue-light response.

Photosynthetic response to temperature

Stomatal opening influences both leaf temperature and the extent of transpiration water loss. A leaf with an effective thickness of 300 μm of primarily water would warm up to a very high temperature if all available solar energy were absorbed and no heat were lost. This heat load is dissipated by emission of long-wave radiation (at about 10,000 nm), by sensible heat loss, and by evaporative (or latent) heat loss (**Figure 2.18**):

1. Radiative heat loss: all objects emit radiation in proportion to their temperature. However, the maximum wavelength is inversely proportional to its temperature, and leaf temperatures are low enough that the wavelength emitted are not visible to the human eye.

2. Sensible heat loss: if the temperature of the leaf is higher than that of the air circulating around the leaf, the heat is convected (transferred) from the leaf to the air.

3. Latent heat loss: because the evaporation of water requires energy, when water evaporates from a leaf (transpiration), it withdraws large amounts of heat from the leaf and cools it.

Sensible heat loss and evaporative heat loss are the most important processes in the regulation of leaf temperature, and the ratio of the two fluxes is called the **Bowen ratio**. In water-stressed crop, partial stomatal closure reduces evaporative cooling and the Bowen ratio is increased. The amount of evaporative heat loss is influenced by the degree to which stomata remain open. Plants with very high Bowen ratios conserve water, but also ensure very high leaf temperatures.

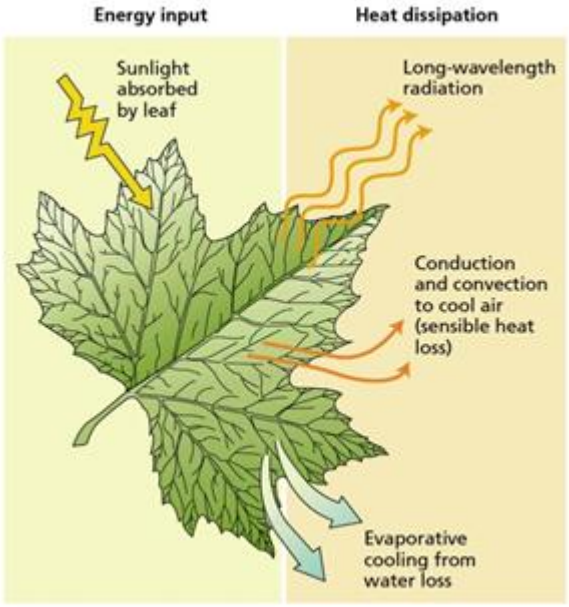


Figure 2.18 The absorption and dissipation of energy from sunlight by the leaf (*source: Taiz L., Zeiger E., 2010*)

There is an optimal temperature for photosynthesis

The highest photosynthetic rates seen in response to increasing temperature represent the **optimal temperature response**. Optimal temperature is the point at which the capacities of the various steps of photosynthesis are optimally balanced, with some of the steps becoming limiting as the temperature decreases or increases. Membrane-bound electron transport processes become unstable at high temperatures, cutting off the supply of reducing power and leading to a sharp overall decrease in photosynthesis. Optimal temperatures have strong genetic (adaptation) and environmental (acclimation) components. Plants of different species growing in habitats with different temperatures have different optimal temperatures for photosynthesis. Plants growing at low temperatures maintain higher photosynthetic rates at low temperatures than plants grown at high temperatures.

Photosynthetic responses to carbon dioxide

In the presence of adequate amounts of light, higher CO2 concentrations support higher photosynthetic rates. The reverse is also true: low CO2 concentrations can limit the amount of photosynthesis in C3 plants. Carbon dioxide is a trace gas in the atmosphere, presently accounting for about 0.039%, or 390 parts per million (ppm), of air. Currently the CO2 concentration of the atmosphere is increasing by about 1 to 3 ppm each year. By 2100 the atmospheric CO2 concentration could reach 600 to 750 ppm unless fossil fuel emission are controlled. Carbon dioxide and methane, play a role similar to that of the glass roof in a greenhouse. The increased CO2 concentration and temperature associated with the greenhouse effect can influence photosynthesis. At current atmospheric CO2 concentrations, photosynthesis in C3 plants is CO2 limited, but this situation could change as atmospheric CO2 concentrations continue to rise. Under laboratory conditions, most C3 plants grow 30 to 60% faster when CO2 concentration is doubled (to 600-750 ppm), and the growth rate becomes limited by the nutrient available to the plant.

Carbon dioxide diffuses through the pore into the substomatal cavity and into the intercellular spaces between mesophyll cells. This portion of the diffusion path of CO2 into the chloroplast is a gaseous phase. The remainder of the diffusion path to the chloroplast is a liquid phase, which begins at the water layer that wets the walls of the mesophyll cells and continue through the plasma membrane, the cytosol, and the chloroplast (**Figure 2.19**). In air of high relative humidity, the diffusion gradient that drives water loss is about 50 times larger than the gradient that drives CO2 uptake. In drier air, this difference can be even larger. Therefore, a decrease in stomatal resistance through the opening of stomata facilitates higher CO2 uptake but is unavoidably accompanied by substantial water loss.

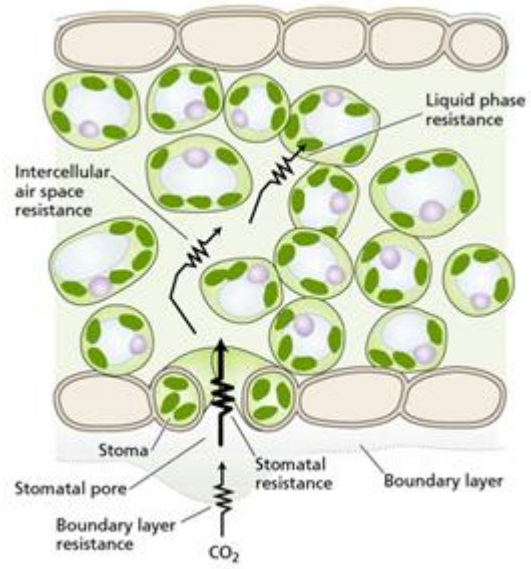


Figure 2.19 Points of resistance to the diffusion of CO2 from outside the leaf to the chloroplasts (*source: Taiz L., Zeiger E., 2010*)

For most leaves, once CO2 has diffused through the stomata, internal CO2 diffusion is rapid, so limitations on photosynthetic performance within the leaf are imposed by factors other than internal CO2 supply. The capacity of leaf tissue for photosynthetic CO2 assimilation depends to a large extent on its rubisco content.

CO2 imposes limitations on photosynthesis

Increasing intracellular CO2 to the concentration at which photosynthesis and respiration balance each other defines the **CO2 compensation point**, at which the net efflux of CO2 from the leaf is zero. This concept is analogous to that of the light compensation point. The CO2 compensation point reflects the balance between photosynthesis and respiration as a function of CO2 concentration, whereas the light compensation point reflects that balance as a function of photon flux under constant O2 concentration.

In C3 plants, increasing atmospheric CO2 above the compensation point stimulates photosynthesis over a wide concentration range. At low to intermediate CO2 concentrations, photosynthesis is limited by the carboxylation capacity of rubisco. At high CO2 concentrations, photosynthesis becomes limited by the capacity of the Calvin-Benson cycle to regenerate the acceptor molecule ribulose 1,5-bisphosphate, which depends on electron transport rates. However, photosynthesis continues to increase with increasing CO2 because carboxylation replaces oxygenation on rubisco.

C4 plants can use water and nitrogen more efficiently than C3 plants can. On the other hand, the additional energy cost of the concentrating mechanism makes C4 plants less efficient in their utilization of light. This is probable one of the reasons that most shade-adapted plants in temperate regions are C3 plants.

The ratio of water loss to CO2 uptake is much lower in CAM plants than it is in either C3 or C4 plants. This is because stomata are primarily open only at night, when lower temperatures and higher humidity contribute to a lower transpiration rate. The main photosynthetic constraints on CAM metabolism is that the capacity to store malic acid is limited, and this limitation restricts the total amount of CO2 uptake.

4. Photosynthesis inhibiting herbicides

The use of herbicides to kill unwanted plants is widespread in modern agriculture. Some herbicides, like dichlorophenyldimethylurea (DCMU, also known as diuron) and paraquat, block photosynthetic electron flow (**Figure 2.20**). DCMU blocks electron flow at the quinone acceptors of photosystem II, by competing for the binding site of plastoquinone that is normally occupied by PQB. Paraquat accepts electrons from the early acceptors of photosystem I and then reacts with oxygen to form superoxide, O2-, a species that is very damaging to chloroplast components, especially lipids.

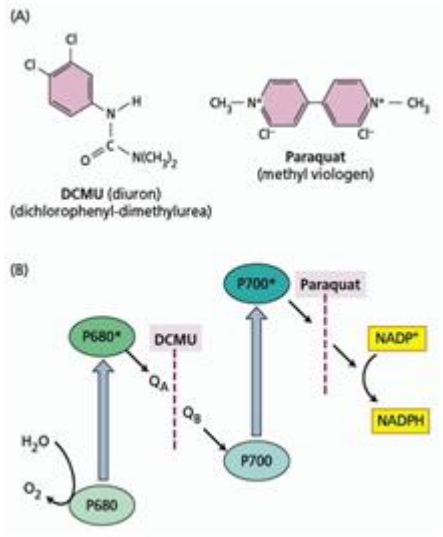


Figure 2.20 Chemical structure and mechanism of action of two important herbicides (*source: Taiz L., Zeiger E., 2010*)

5. Secondary metabolites in plant defences

The sum of all of the chemical reactions that take place in an organism is called metabolism. Most of that carbon, nitrogen, and energy ends up in molecules that are common to all cells and are required for the proper functioning of cells and organisms. These molecules, e.g., lipids, proteins, nucleic acids, and carbohydrates, are called **primary metabolites**. Unlike animals, however, most plants divert a significant proportion of assimilated carbon and energy to the synthesis of organic molecules that may have no obvious role in normal cell function. These molecules are known as **secondary metabolites**.

The distinction between primary and secondary metabolites is not always easily made. At the biosynthetic level, primary and secondary metabolites share many of the same intermediates and are derived from the same core metabolic pathways. Secondary metabolites generally, but not always, occur in relatively low quantities and their production may be widespread or restricted to particular families, genera, or even species. They were known, however, to have significant economic and medicinal value and were thus of more than a passing interest to natural products chemists. In recent years, however, it has become increasingly evident that many natural products do have significant ecological functions, such as protection against microbial or insect attack.

Secondary metabolites

For many years the adaptive significance of most secondary metabolites was unknown. These compounds were thought to be simply functionless end products of metabolism, or metabolic wastes. Today we know that many secondary metabolites have important ecological functions in plants:

- They protect plants against being eaten by herbivores and against being infected by microbial pathogens.
- They serve as attractants (odor, color, taste) for pollinators and seed-dispersing animals.
- They function as agents of plant-plant competition and plant-microbe symbioses.

The ability of plants to compete and survive is therefore profoundly affected by the ecological functions of their secondary metabolites.

Secondary metabolism is also relevant to agriculture. The very defensive compounds that increase the reproductive fitness of plants by warding off fungi, bacteria, and herbivores may also make them undesirable as food for humans. Many important crop plants have been artificially selected to produce relatively low levels of these compounds (which, of course, can make them more susceptible to insects and disease).

Plant secondary metabolites can be divided into three chemically distinct groups: **terpenes, phenolics, and nitrogen-containing compounds**.

Terpenes

The terpenes, or terpenoids, constitute the largest class of secondary metabolites. Most of the diverse substances of this class are insoluble in water. Certain terpenes have well-characterized functions in plant growth or development and so can be considered primary rather than secondary metabolites. For example, the gibberellins, an important group of plant hormones, are diterpenes. Brassinosteroids, another class of plant hormones with growth-regulating functions, originate from triterpenes. The vast majority of terpenes, however, are secondary metabolites presumed to be involved in *plant defenses*.

Terpenes are toxins and feeding deterrents to many herbivorous insects and mammals; thus they appear to play important defensive roles in the plant kingdom. For example, monoterpene esters called *pyrethroids*, found in the leaves and flowers of *Chrysanthemum* species, show striking insecticidal activity. Both natural and synthetic pyrethroids are popular ingredients in commercial insecticides because of their low persistence in the environment and their negligible toxicity to mammals. In conifers such as pine and fir, monoterpenes accumulate in resin ducts found in the needles, twigs, and trunk. These compounds are toxic to numerous insects, including bark beetles, which are serious pests of conifer species throughout the world. Many plants contain mixtures of volatile monoterpenes and sesquiterpenes, called essential oils, that lend a characteristic odor to their foliage. Peppermint, lemon, basil, and sage are examples of plants that contain essential oils. The chief monoterpene constituent of lemon oil is limonene; that of peppermint oil is menthol (**Figure 2.21**). Essential oils have well-known insect repellent properties.

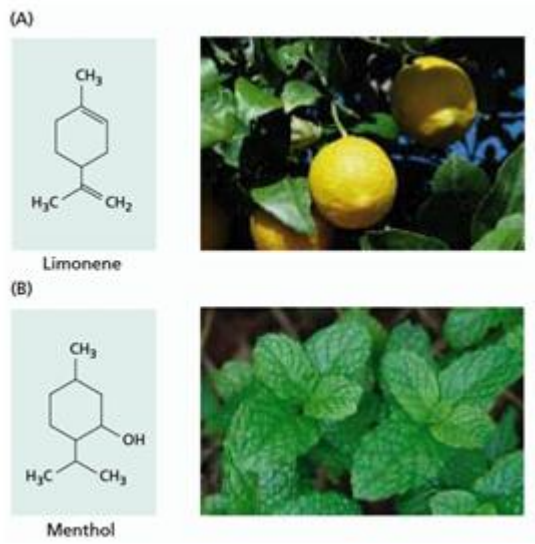


Figure 2.21 Structures of limonene (A) and menthol (B): these two well-known monoterpenes serve as defenses against insects and other organisms (*source: Taiz L., Zeiger E., 2010*)

They are frequently found in glandular hairs that project outward from the epidermis and serve to "advertise" the toxicity of the plant, repelling potential herbivores even before they take a trial bite. Triterpenes that defend plants against vertebrate herbivores include *cardenolides and saponins*. Cardenolides are glycosides (compounds containing an attached sugar or sugars) that taste bitter and are extremely toxic to higher animals. Saponins are steroid and triterpene glycosides, so named because of their soaplike properties. The presence of both lipid-soluble (the steroid or triterpene) and water-soluble (the sugar) elements in one molecule gives saponins detergent properties.

Phenolic compounds

Plants produce a large variety of secondary compounds that contain a phenol group: a hydroxyl functional group on an aromatic ring. These substances are classified as *phenolic compounds, or phenolics*. Plant phenolics are a chemically heterogeneous group of nearly 10,000 individual compounds: Some are soluble only in organic solvents, some are water-soluble carboxylic acids and glycosides, and others are large, insoluble polymers. In keeping with their chemical diversity, phenolics play a variety of roles in the plant. Many serve as defenses against herbivores and pathogens. Others function in mechanical support, in attracting pollinators and fruit dispersers, in absorbing harmful ultraviolet radiation, or in reducing the growth of nearby competing plants.

The colored pigments of plants provide visual cues that help to attract pollinators and seed dispersers. These pigments are of two principal types: *carotenoids and flavonoids*. Carotenoids are yellow, orange, and red terpenoid compounds that also serve as accessory pigments in photosynthesis. The flavonoids also include a wide range of colored substances. The most widespread group of pigmented flavonoids is the *anthocyanins*, which are responsible for most of the red, pink, purple, and blue colors observed in flowers and fruits. Two other groups of flavonoids found in flowers are *flavones and flavonols*. These flavonoids generally absorb light at shorter wavelengths than do anthocyanins, so they are not visible to the human eye. However, insects such as bees, which see farther into the ultraviolet range of the spectrum than humans do, may respond to flavones and flavonols as visual attractant cues. *Isoflavonoids*, which are found mostly in legumes, have several different biological activities. Some, such as rotenone, can be used effectively as insecticides, pesticides (e.g., as rat poison), and piscicides (fish poisons). Other isoflavones have anti-estrogenic effects; for example, sheep grazing on clover rich in isoflavonoids often suffer from infertility. The ring system of isoflavones has a three-dimensional structure similar to that of steroids, allowing these substances to bind to estrogen receptors. Isoflavones may also be responsible for the anticancer benefits of foods prepared from soybeans.

A second category of plant phenolic polymers with defensive properties, besides lignin, is the *tannins*. They are general toxins that can reduce the growth and survival of many herbivores when added to their diets. In addition, tannins act as feeding repellents to a great variety of animals. Mammals such as cattle, deer, and apes characteristically avoid plants or parts of plants with high tannin contents. Unripe fruits, for instance, frequently have very high tannin levels, which deter feeding on the fruits until their seeds are mature enough for dispersal. Herbivores that habitually feed on tannin-rich plant material appear to possess some interesting adaptations to remove tannins from their digestive systems. Plant tannins also serve as defenses against microorganisms.

From leaves, roots, and decaying litter, plants release a variety of primary and secondary metabolites into the environment. The release of secondary compounds by one plant that have an effect on neighboring plants is referred to as **allelopathy**. If a plant can reduce the growth of nearby plants by releasing chemicals into the soil, it may increase its access to light, water, and nutrients and thus its evolutionary fitness. Allelopathy is currently of great interest because of its potential agricultural applications. Reductions in crop yields caused by weeds or residues from the previous crop may in some cases be a result of allelopathy. An exciting future prospect is the development of crop plants genetically engineered to be allelopathic to weeds.

Nitrogen-containing compounds

A large variety of plant secondary metabolites have nitrogen as part of their structure. Included in this category are such well-known antiherbivore defenses as *alkaloids* and *cyanogenic glycosides*, which are of considerable interest because of their toxicity to humans as well as their medicinal properties. Most nitrogenous secondary metabolites are synthesized from common amino acids.

The *alkaloids* are a large family of more than 15,000 nitrogen-containing secondary metabolites. They are found in approximately 20% of vascular plant species. As a group, alkaloids are best known for their striking pharmacological effects on vertebrate animals. Alkaloids are usually synthesized from one of a few common amino acids – in particular, lysine, tyrosine, or tryptophan. However, the carbon skeleton of some alkaloids contains a component derived from the terpene pathway. Several different types, including nicotine and its relatives (**Figure 2.22**), are derived from ornithine, an intermediate in arginine biosynthesis. The B vitamin nicotinic acid (niacin) is a precursor of the pyridine (six-membered) ring of this alkaloid. Alkaloids were once thought to be nitrogenous wastes (analogous to urea and uric acid in animals), nitrogen storage compounds, or growth regulators, but there is little evidence to support any of these functions. Most alkaloids are now believed to function as defenses against herbivores, especially mammals, because of their general toxicity and deterrence capability.

Various nitrogenous protective compounds other than alkaloids are found in plants. Two groups of these substances – cyanogenic glycosides and glucosinolates – are not themselves toxic but are readily broken down to give off poisons, some of which are volatile, when the plant is crushed. *Cyanogenic glycosides* release the well-known poisonous gas hydrogen cyanide (HCN). The presence of cyanogenic glycosides deters feeding by insects and other herbivores such as snails and slugs. As with other classes of secondary metabolites, however, some herbivores have adapted to feed on cyanogenic plants and can tolerate large doses of HCN.

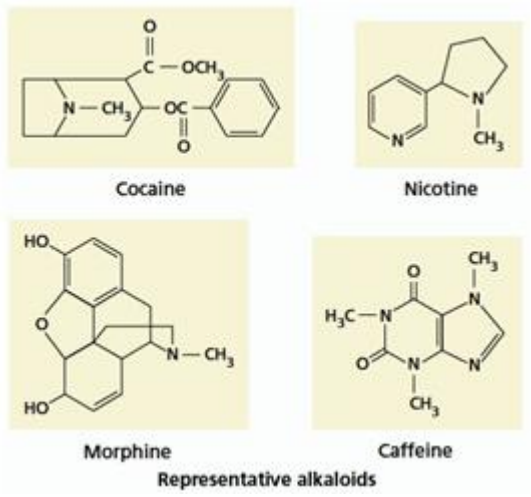


Figure 2.22 Examples of alkaloids, a diverse group of secondary metabolites that contain nitrogen (*source: Taiz L., Zeiger E., 2010*)

A second class of plant glycosides, called the *glucosinolates*, or mustard oil glycosides, break down to release defensive substances. Found principally in the *Brassicaceae* and related plant families, glucosinolates break down to produce the compounds responsible for the smell and taste of vegetables such as cabbage, broccoli, and radishes. Glucosinolate breakdown is catalyzed by a hydrolytic enzyme, called a thioglucosidase or myrosinase, that cleaves glucose from its bond with the sulfur atom. These defensive products function as toxins and herbivore repellents. Like cyanogenic glycosides, glucosinolates are stored in the intact plant separately from the enzymes that hydrolyze them, and they are brought into contact with these enzymes only when the plant is crushed.

Plants and animals incorporate the same 20 amino acids into their proteins. However, many plants also contain unusual amino acids, called *nonprotein amino acids*, that are not incorporated into proteins. Instead, these amino acids are present in the free form and act as defensive substances. Many nonprotein amino acids are very similar to common protein amino acids. Nonprotein amino acids exert their toxicity in various ways. Some block the synthesis or uptake of protein amino acids. Others, such as canavanine, can be mistakenly incorporated into proteins. After ingestion by an herbivore, canavanine is recognized by the enzyme that normally binds arginine to the arginine transfer RNA molecule, so it becomes incorporated into herbivore proteins in place of arginine. Plants that synthesize nonprotein amino acids are not susceptible to the toxicity of these compounds.

Induced plant defenses against insect herbivores

Plants have developed a wide variety of defensive strategies against insect herbivory. These strategies can be divided into two categories: constitutive defenses and induced defenses. *Constitutive defenses* are defensive mechanisms that are always present in the plant. They are often species-specific and may exist as stored compounds, conjugated compounds (to reduce toxicity), or precursors of active compounds that can easily be activated if the plant is damaged. Most of the defensive secondary compounds are constitutive defenses. *Induced defenses* are initiated only after actual damage occurs. They include the production of defensive proteins such as lectins and protease inhibitors as well as the production of toxic secondary metabolites. In principle, induced defenses require a smaller investment of plant resources than constitutive defenses, but they must be activated quickly to be effective.

Plants can recognize specific components of insect saliva

The plant response to damage by insect herbivores involves both a wound response and the recognition of certain insect-derived compounds referred to as elicitors. Although repeated mechanical wounding can induce responses similar to those caused by insect herbivory in some plants, certain molecules in insect saliva can serve as enhancers of this stimulus. In addition, such insect-derived elicitors can trigger signaling pathways systemically, thereby initiating defensive responses in distant regions of the plant in anticipation of further damage. After being regurgitated by an insect, elicitors become part of its saliva and are thus applied to the feeding site during herbivory. Plants then recognize these elicitors and activate a complex signal transduction pathway that induces their defenses.

Jasmonic acid activates many defensive responses

A major signaling pathway involved in most plant defenses against insect herbivores is the octadecanoid pathway, which leads to the production of a plant hormone called *jasmonic acid* (JA or jasmonate). Jasmonic acid levels rise steeply in response to insect herbivore damage and trigger the production of many proteins involved in plant defenses. Jasmonic acid is synthesized from linolenic acid, which is released from plant membrane lipids. Two organelles participate in jasmonic acid biosynthesis: the chloroplast and the peroxisome. Jasmonic acid is known to induce the transcription of a host of genes involved in defensive metabolism. Among the genes it induces are those that encode key enzymes in all the major pathways for secondary metabolite biosynthesis. Several other signaling compounds – including ethylene, salicylic acid, and methyl salicylate – are also induced by insect herbivory. In many cases, the concerted action of these signaling compounds is necessary for the full activation of induced defenses.

Some plant proteins inhibit herbivore digestion

Among the diverse components of plant defensive arsenals induced by jasmonic acid are proteins that interfere with herbivore digestion. For example, some legumes synthesize a-amylase inhibitors that block the action of the starch-digesting enzyme α-amylase. Other plant species produce lectins, defensive proteins that bind to carbohydrates or carbohydrate-containing proteins. After ingestion by an herbivore, lectins bind to the epithelial cells lining the digestive tract and interfere with nutrient absorption. The best-known antidigestive proteins in plants are the protease inhibitors. Found in legumes, tomatoes, and other plants, these substances block the action of herbivore proteolytic enzymes (proteases).

Herbivore-induced volatiles have complex ecological functions

The induction and release of volatile organic compounds, also called volatiles, in response to insect herbivore damage provides an excellent example of the complex ecological functions of secondary metabolites in nature. The combination of molecules emitted is often specific for each insect herbivore species and typically includes representatives from the three major classes of secondary metabolites: terpenes, phenolics, and alkaloids. Additionally, in response to mechanical damage, all plants emit lipid-derived products such as green-leaf volatiles, a mixture of six-carbon aldehydes, alcohols, and esters. The ecological functions of these volatiles are manifold. In many cases, they attract natural enemies – predators or parasites – of the attacking insect herbivore that utilize the volatiles as cues to find their prey or hosts for their offspring. Volatiles released by the leaf during moth oviposition (egg laying) can act as repellents to other female moths, thereby preventing further egg deposition and herbivory. In addition, many of these compounds, although volatile, remain attached to the surface of the leaf and serve as feeding deterrents because of their taste.

Plant defenses against pathogens

Plants are continuously exposed to a diverse array of pathogens. To be successful, these pathogens have developed various strategies to invade their host plants. Some penetrate the cuticle and cell wall directly by secreting lytic enzymes, which digest these mechanical barriers. Others enter the plant through natural openings like stomata and lenticels. A third category invades the plant through wounding sites, for example those caused by insect herbivores. Additionally, many viruses, as well as other types of pathogens are transferred by insect herbivores, which serve as vectors, and invade the plant from the insect feeding site. Phloem feeders such as whiteflies and aphids deposit pathogens directly into the vascular system, from which they can easily spread throughout the plant.

Some antimicrobial compounds are synthesized before pathogen attack

Several classes of secondary metabolites have strong antimicrobial activity when tested in vitro; thus they have been proposed to function as defenses against pathogens in the intact plant. Among these are saponins, a group of triterpenes thought to disrupt fungal membranes by binding to sterols. Experiments utilizing genetic approaches have demonstrated the role of saponins in defense against pathogens of oat. Mutant oat lines with reduced saponin levels had much less resistance to fungal pathogens than did wild-type oats. Interestingly, one fungal strain that normally grows on oats was able to detoxify one of the principal saponins in the plant.

Infection induces additional antipathogen defenses

After being infected by a pathogen, plants deploy a broad spectrum of defenses against the invading microbes. A common defense is the **hypersensitive response**, in which cells immediately surrounding the infection site die rapidly, depriving the pathogen of nutrients and preventing its spread. After a successful hypersensitive

response, a small region of dead tissue is left at the site of the attempted invasion, but the rest of the plant is unaffected. The hypersensitive response is often preceded by the rapid accumulation of *reactive oxygen species and nitric oxide* (NO). Cells in the vicinity of the infection synthesize a burst of toxic compounds formed by the reduction of molecular oxygen. Active oxygen species may contribute to host cell death as part of the hypersensitive response or act to kill the pathogen directly. Another defensive response to infection is the formation of hydrolytic enzymes that attack the cell wall of the pathogen. An assortment of glucanases, chitinases, and other hydrolases are induced by fungal invasion. These hydrolytic enzymes belong to a group of proteins that are closely associated with pathogen infection and so are known as *pathogenesis-related (PR) proteins.*

Phytoalexins often increase after pathogen attack

Perhaps the best-studied response of plants to bacterial or fungal invasion is the synthesis of *phytoalexins*. Phytoalexins are a chemically diverse group of secondary metabolites with strong antimicrobial activity that accumulate around the site of an infection. Phytoalexin production appears to be a common mechanism of resistance to pathogenic microbes in a wide range of plants. However, different plant families employ different types of secondary products as phytoalexins. For example, in leguminous plants, such as alfalfa and soybean, isoflavonoids are common phytoalexins, whereas in solanaceous plants, such as potato, tobacco, and tomato, various sesquiterpenes are produced as phytoalexins. Phytoalexins are generally undetectable in the plant before infection, but they are synthesized very rapidly after microbial attack. The point of control for the activation of these biosynthetic pathways is usually the initiation of gene transcription. Thus plants do not appear to store any of the enzymatic machinery required for phytoalexin synthesis. Instead, soon after microbial invasion, they begin transcribing and translating the appropriate mRNAs and synthesizing the enzymes *de novo.*

Some plants recognize specific pathogen-derived substances

Within a species, individual plants often differ greatly in their resistance to microbial pathogens. These differences often lie in the speed and intensity of a plant's reactions. Resistant plants respond more rapidly and more vigorously to pathogens than do susceptible plants. Hence it is important to learn how plants sense the presence of pathogens and initiate defensive responses. A first line of resistance is provided by a system that recognizes broad categories of pathogens. Plants have a variety of receptors that recognize so-called **microbe-associated general molecular patterns (MAMPs)**. These elicitors are evolutionary conserved pathogen-derived molecules such as structural elements from the fungal cell wall or the bacterial flagellum. MAMPs are recognized by specific receptors, which then activate specific plant defensive responses, including massive phytoalexin production. The effectiveness of these MAMP receptors (or pattern recognition receptors) is amazing, considering the fact that with one receptor, a plant can recognize a complete taxonomic group that features a particular MAMP. For example, the flagellin (flg22) receptor FLS2 enables the plant to recognize all mobile (flagellated) bacteria. Similarly, the as yet uncharacterized receptor for pep13 enables plants to recognize all oomycete pathogens. Consequently, those pathogens cannot cause disease. This form of defensive strategy is also referred to as **innate immunity.**

A single encounter with a pathogen may increase resistance to future attacks

When a plant survives infection by a pathogen at one site, it often develops increased resistance to subsequent attacks at sites throughout the plant and enjoys protection against a wide range of pathogenic species. This phenomenon, called **systemic acquired resistance (SAR)** (**Figure 2.23**), develops over several days following initial infection. Systemic acquired resistance appears to result from increased levels of certain PR proteins that we have already mentioned, including chitinases and other hydrolytic enzymes. Although the mechanism of SAR induction is still unknown, one of the endogenous signals involved is likely to be *salicylic acid*. This benzoic acid derivative accumulates dramatically in the zone of infection after the initial attack, and it is thought to establish SAR in other parts of the plant. Another compound that accumulates at the site of infection and may play a role in SAR is H2O2. However, like salicylic acid, H2O2 is unlikely to function as a long-distance signal.

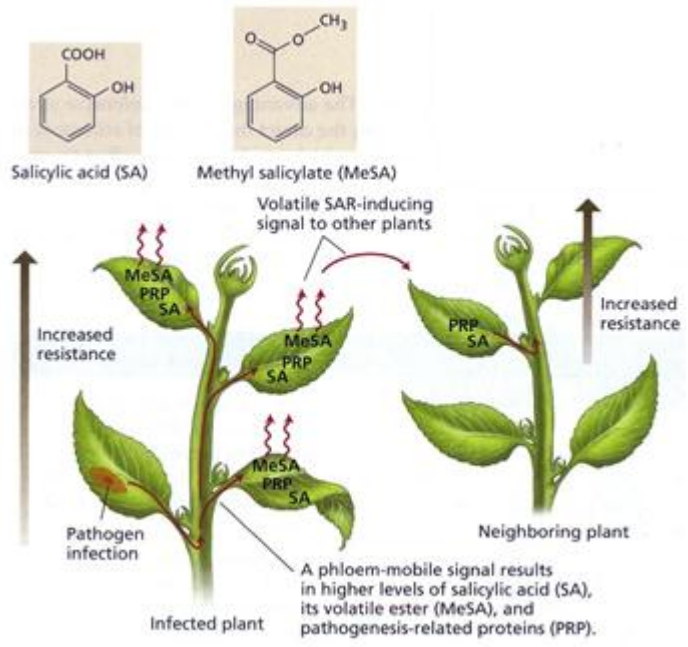


Figure 2.23 Initial pathogen infection may increase resistance to future pathogen attack through development of systemic acquired resistance (SAR) (*source: Taiz L., Zeiger E., 2010*)

Interactions of plants with non-pathogenic bacteria can trigger induced systemic resistance

In contrast to SAR, which occurs as a consequence of actual pathogen infection, **induced systemic resistance (ISR)** is activated by nonpathogenic microbes (**Figure 2.24**). Colonialization of the root zone by rhizobacteria, for example, not only stimulates the formation of root nodules, but also initiates a signaling cascade throughout the plant. As a consequence of this signaling cascade, which involves JA and ethylene, protective measures are activated throughout the plant, resulting in an enhanced mode of preparedness against pathogen attack. This form of systemic defense activation does not involve salicylic acid as a signaling compound and does not induce the accumulation of typical PR proteins. While certain defensive measures are immediately put in place by ISR, other defensive responses are initiated only after actual pathogen infection, resulting in a faster and stronger response. The advantage of this defensive strategy lies in reducing the direct investment of resources in defensive measures, which would otherwise affect the performance of the plant, resulting, for example, in reduced growth and yield.

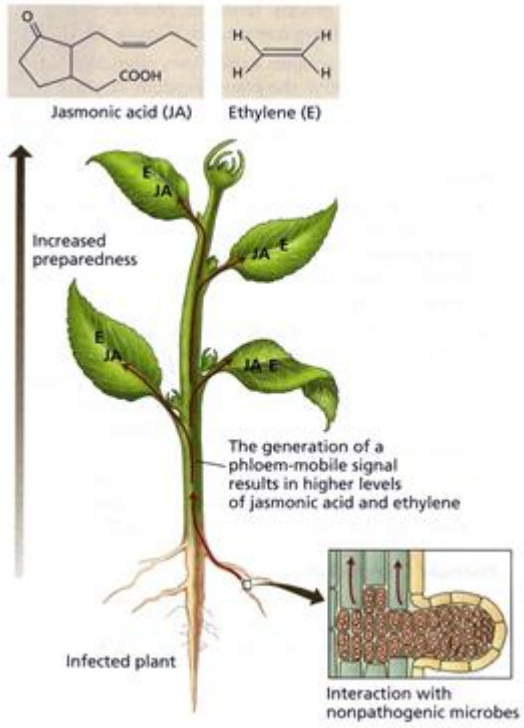


Figure 2.24 Exposure to nonpathogenic microorganisms may increase resistance to future pathogen attack through development of induced systemic resistance (ISR) (*source: Taiz L., Zeiger E., 2010*)

Chapter 4. Physiology of plant growth and development

1. Cell wall biogenesis and expansion

The structure and biosynthesis of plant cell wall

The cell walls of prokaryotes, fungi, algae, and plants are distinctive from each other in chemical composition and microscopic structure, yet they all serve two common primary functions: regulating cell volume and determining cell shape. Because of these diverse functions, the structure and composition of plant cell walls are complex and variable.

In addition to these biological functions, the plant cell wall is important in human economics. As a natural product, the plant cell wall is used commercially in the form of paper, textiles, fibers (cotton, flax, hemp, and others), charcoal, lumber, and other wood products. Another major use of plant cell walls is in the form of extracted polysaccharides that have been modified to make plastics, films, coatings, adhesives, gels, and thickeners in a huge variety of products.

As the most abundant reservoir of organic carbon in nature, the plant cell wall also takes part in the processes of carbon flow through ecosystems.The organic substances that make up humus in the soil and that enhance soil structure and fertility are derived from cell walls. Finally, as an important source of roughage in our diet, the plant cell wall is a significant factor in human health and nutrition.

The architecture, mechanics and function of plants depend on the structure of the cell wall

In stained sections of plant tissues reveal that the cell wall is not uniform, but varies greatly in appearance and composition in different cell types. Cell walls of the cortical parenchyma are generally thin and have few distinguishing features. In contrast, the walls of some specialized cells, such as epidermal cells, collenchyma, phloem fibers, xylem tracheary elements, and other forms of sclerenchyma have thicker, multilayered walls. Often these walls are intricately sculpted and are impregnated with specific substances, such as lignin, cutin, suberin, waxes, silica, or structural proteins.

Despite this diversity in cell wall morphology, cell walls commonly are classified into two major types: *primary walls and secondary walls*. Primary walls are formed by growing cells and are usually considered to be relatively unspecialized and similar in molecular architecture in all cell types. Nevertheless, the ultrastructure of primary walls also shows wide variation. Some primary walls, such as those of the onion bulb parenchyma, are very thin (100 nm) and architecturally simple. Other primary walls, such as those found in collenchyma or in the epidermis, may be much thicker and consist of multiple layers (**Figure 3.1**).

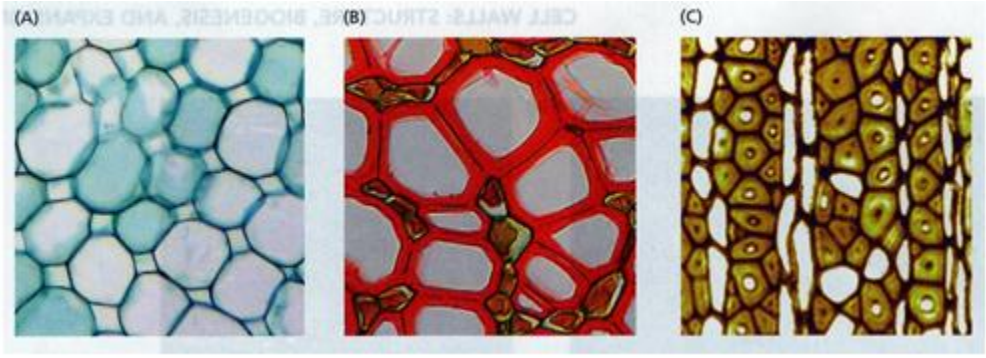


Figure 3.1 Diversity of plant cell wall structure. (A) primary, and (B)-(C) secondary cell walls (*source: Taiz L., Zeiger E., 2010*)

Secondary walls are the cell walls that form after cell growth (enlargement) has ceased. Secondary walls may become highly specialized in structure and composition, reflecting the differentiated state of the cell. Xylem

cells, such as those found in wood, are notable for possessing highly thickened secondary walls that are strengthened by lignin.

A thin layer of material, the *middle lamella* (plural lamellae), can usually be seen at the junction where the walls of neighboring cells come into contact. The composition of the middle lamella differs from the rest of the wall in that it is high in pectin and contains different proteins compared with the bulk of the wall. Its origin can be traced to the cell plate that formed during cell division.

The cell wall is usually penetrated by tiny membrane-lined channels, called *plasmodesmata* (singular plasmodesma), which connect neighboring cells. Plasmodesmata function in communication between cells, by allowing passive transport of small molecules and active transport of proteins and nucleic acids between the cytoplasms of adjacent cells.

Primary cell wall is a network of cellulose microfibrils embedded in a matrix of hemicelluloses, pectins, and structural proteins

In primary cell walls, cellulose microfibrils are embedded in a highly hydrated *matrix*. This structure provides both strength and flexibility. In the case of cell walls, the matrix consists of two major groups of polysaccharides, usually called *hemicelluloses and pectins*, plus a small amount of structural protein.

Cellulose microfibrils are relatively stiff structures that contribute to the strength and structural bias of the cell wall. The individual glucans that make up the microfibril are closely aligned and bonded to each other to make a highly ordered (crystalline) ribbon that excludes water and is relatively inaccessible to enzymatic attack. As a result, cellulose is very strong and very stable and resists degradation (**Figure 3.2**).

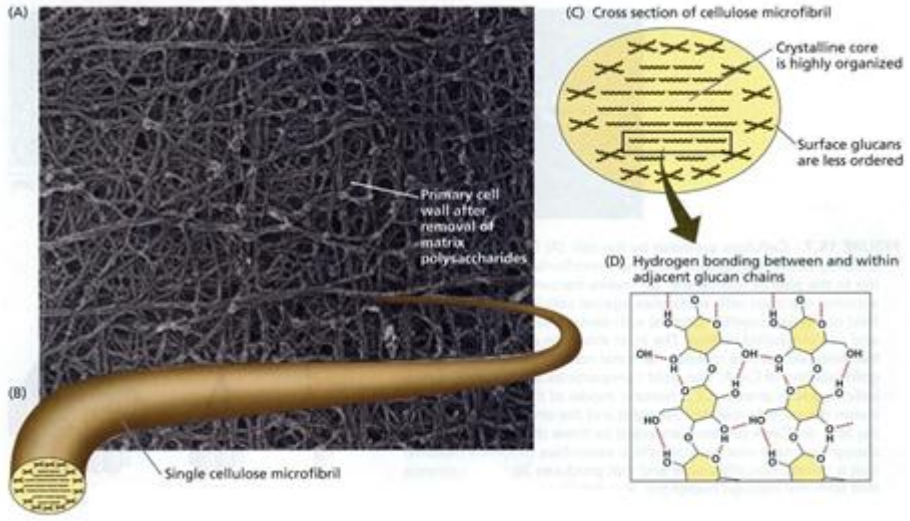


Figure 3.2 A structural model of a cellulose microfibril (*source: Taiz L., Zeiger E., 2010*)

Hemicelluloses are flexible polysaccharides that characteristically bind to the surface of cellulose. They may form tethers that bind cellulose microfibrils together into a cohesive network, or they may act as a slippery coating to prevent direct microfibril–microfibril contact. Another term for these molecules is cross-linking glucans. The term hemicellulose includes several different kinds of polysaccharides.

Pectins form a hydrated gel phase in which the cellulose–hemicellulose network is embedded. They act as hydrophilic filler, to prevent aggregation and collapse of the cellulose network. They also determine the porosity of the cell wall to macromolecules. Like hemicelluloses, pectins include several different kinds of polysaccharides.

The precise role of wall *structural proteins* is uncertain, but they may add mechanical strength to the wall and assist in the proper assembly of other wall components. The primary wall is composed of approximately 25% cellulose, 25% hemicelluloses, and 35% pectins, with perhaps 1 to 8% structural protein, on a dry-weight basis. However, large deviations from these values may be found.

The primary wall also contains much *water*. This water is located mostly in the matrix, which is perhaps 75 to 80% water. The hydration state of the matrix is an important determinant of the physical properties of the wall; for example, removal of water makes the wall stiffer and less extensible.

Secondary walls in woody tissues contain more cellulose, xylans, and lignin

After wall expansion ceases, cells sometimes continue to synthesize a wall, known as a *secondary wall*. Secondary walls are often quite thick, as in tracheids, fibers, and other cells that serve in mechanical support of the plant. Often such secondary walls are multilayered and differ in structure and composition from the primary wall. For example, the secondary walls in wood contain xylans rather than xyloglucans, as well as a higher proportion of cellulose. The orientation of the cellulose microfibrils may be more neatly aligned parallel to each other in secondary walls than in primary walls. Secondary walls are often (but not always) impregnated with lignin.

Lignin is a phenolic polymer with a complex, irregular pattern of linkages that link the aromatic alcohol subunits together. These subunits are synthesized from phenylalanine and are secreted to the wall, where they are oxidized in place by the enzymes peroxidase and laccase. As lignin forms in the wall, it displaces water from the matrix and forms a hydrophobic network that bonds tightly to cellulose and prevents wall enlargement.

Cell wall elongation and degradation

During plant cell enlargement, new wall polymers are continuously synthesized and secreted at the same time that the preexisting wall is expanding.

Microfibril orientation influences growth directionality of cells with diffuse growth

During growth, the loosened cell wall is extended by physical forces generated from cell turgor pressure. Turgor pressure creates an outward-directed force, equal in all directions. The directionality of growth is determined largely by the structure of the cell wall – in particular, the orientation of cellulose microfibrils.

When cells first form in the meristem, they are isodiametric; that is, they have equal diameters in all directions. If the orientation of cellulose microfibrils in the primary cell wall were *isotropic* (randomly arranged), the cell would grow equally in all directions, expanding radially to generate a sphere (**Figure 3.3**). In most plant cell walls, however, the arrangement of cellulose microfibrils is *anisotropic* (nonrandom), or aligned in a preferred direction.

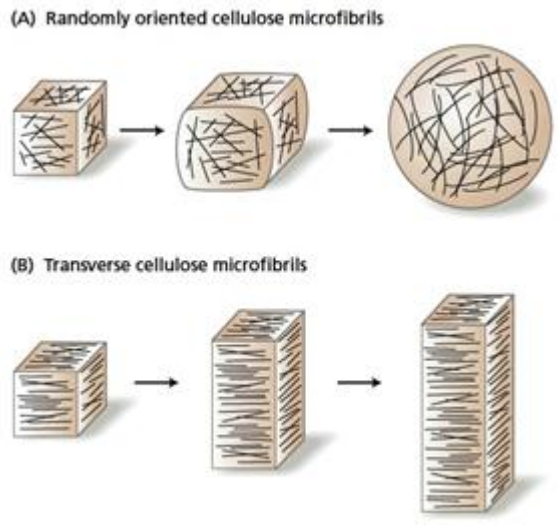


Figure 3.3 The orientation of newly deposited cellulose microfibrils determines the direction of cell expansion (*source: Taiz L., Zeiger E., 2010*)

Acid-induced cell wall extension is characteristic of primary walls and is mediated by the protein expansin

An important characteristic of growing cell walls is that they extend much faster at acidic pH than at neutral pH. This phenomenon is called *acid growth*. In living cells, acid growth is evident when growing cells are treated

with acid buffers or with the drug fusicoccin, which induces acidification of the cell wall solution by activating an H+-ATPase in the plasma membrane. Auxin-induced growth is also associated with wall acidification, but it is probably not sufficient to account for the entire growth induction by this hormone, and other wall-loosening processes may be involved.

The idea that proteins are required for acid growth was confirmed in reconstitution experiments, in which heat inactivated walls were restored to nearly full acid growth responsiveness by addition of proteins extracted from growing walls. The active components proved to be a group of proteins that were named *expansins*. These proteins catalyze the pH-dependent extension and stress relaxation of cell walls.

Plant cell walls play a major role in carbon flow through ecosystems

Most plant cell walls are constructed in a way to resist enzymatic digestion – a defense against pathogen invasion – and so the recycling of the carbon and energy locked in the cell wall is mostly carried out by saprophytic fungi and bacteria armed with a suite of specialized enzymes capable of digesting cell walls. Some animals, such as ruminants and termites, are also able to partake in this fibrous feast, with the aid of gut microbes similarly equipped with cell-wall digesting enzymes. The organic substances that make up humus in the soil and that enhance soil structure and fertility are derived from cell wall residues – one of many legacies of plants to their environment. Finally, as the major source of dietary fiber, the plant cell wall is a significant factor in human health and nutrition.

Cell wall degradation and plant defense

The plant cell wall is not simply an inert, static exoskeleton. In addition to acting as a mechanical restraint, the wall serves as an extracellular matrix that interacts with cell surface proteins, providing positional and developmental information. It contains numerous enzymes and smaller molecules that are biologically active and that can modify the physical properties of the wall, sometimes within seconds. In some cases, wall-derived molecules can also act as signals to inform the cell of environmental conditions, such as the presence of pathogens. This is an important aspect of the defense response of plants.

Walls may also be substantially modified long after growth has ceased. For instance, the cell wall may be selectively degraded, such as occurs in ripening fruit or in the endosperm or cotyledons of germinating seeds. In cells that make up the abscission zones of leaves and fruits, the middle lamella is digested, with the result that the cells become unglued and separate. Cells may also separate selectively during the formation of intercellular air spaces, during the emergence of the root from germinating seeds, and during other developmental processes. Plant cells may also modify their walls during pathogen attack as a form of defense.

2. Overview of plant growth and development

The development of a mature plant from a single fertilized egg follows a precise and highly ordered succession of events. The fertilized egg cell, or zygote, divides, grows, and differentiates into increasingly complex tissues and organs. In the end, these events give rise to the complex organization of a mature plant that flowers, bears fruit, senesces, and eventually dies. These events, along with their underlying genetic programs and biochemistry, and the many other factors that contribute to an orderly progression through the life cycle, constitute development.

The meaning of the terms growth, differentiation and development

Three terms routinely used to describe various changes that a plant undergoes during its life cycle are **growth, differentiation, and development**.

Growth is an irreversible increase in volume or size

Growth is a quantitative term, related only to changes in size and mass. For cells, growth is simply an irreversible increase in volume. For tissues and organs, growth normally reflects an increase in both cell number and cell size.

Growth can be assessed by a variety of *quantitative measures*. Growth of cells such as bacteria or algae in culture, for example, is commonly measured as the fresh weight, cell number or packed cell volume in a centrifuge tube. For higher plants, however, fresh weight is not always a reliable measure. Most plant tissues are approximately 80 percent water, but water content is highly variable and fresh weight will fluctuate widely with

changes in ambient moisture and the water status of the plant. Dry weight, determined after drying the material to a constant weight, is a measure of the amount of protoplasm or dry matter (i.e., everything but the water). Dry weight is used more often than fresh weight, but even dry weight can be misleading as a measure of growth in certain situations. Consider the example of a pea seed that is germinated in darkness (**Figure 3.4**). In darkness, the embryo in the seed will begin to grow and produce a shoot axis that may reach 25 to 30 cm in length. Although we intuitively sense that considerable growth has occurred, the total dry weight of the seedling plus the seed will actually decrease compared with the dry weight of the seed alone prior to germination. The dry weight decreases in this case because some of the carbon stored in the respiring seed is lost as carbon dioxide. In a situation such as this, either fresh weight or the length of the seedling axis would be a better measure of growth. Length, and perhaps width, would also be suitable measures for an expanding leaf. There is not any special universal measure unit to characterize plant growth.

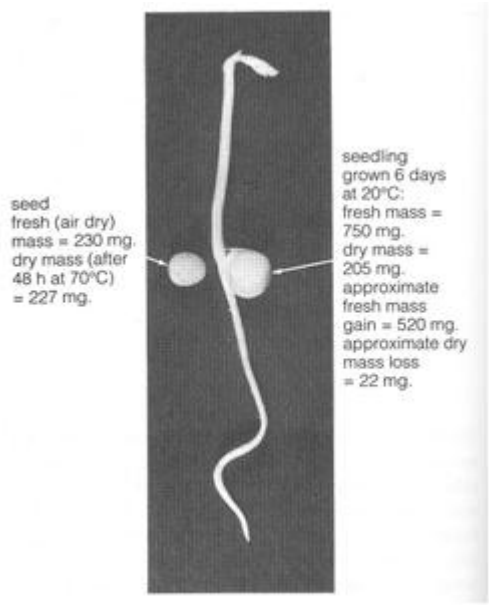


Figure 3.4 Changes in fresh and dry biomass of pea seed as it develops into seedling in darkness (*source: Salisbury F.B., Ross C.W., 1992*)

It should be obvious that many parameters could be invoked to measure growth, dependent to some extent on the needs of the observer. Whatever the measure, however, all attempts to quantify growth reflect a fundamental understanding that growth is an *irreversible increase in volume or size*.

Differentiation refers to qualitative changes that normally accompany growth

Differentiation occurs when cells assume different anatomical characteristics and functions, or form patterns. Differentiation begins in the earliest stages of development, such as, when division of the zygote gives rise to cells that are destined to become either root or shoot. Later, unspecialized parenchyma cells may differentiate into more specialized cells such as xylem vessels or phloem sieve tubes, each with a distinct morphology and unique function.

Differentiation is a two-way street and is not determined so much by cell lineage as by cell position with respect to neighboring cells. Thus, even though some plant cells may appear to be highly differentiated or specialized, they may often be stimulated to revert to a more embryonic form. For example, cells isolated from the center of a tobacco stem or a soybean cotyledon and cultured on an artificial medium may be stimulated to reinitiate cell division, to grow as undifferentiated *callus tissue*, and eventually to give rise to a new plant (**Figure 3.5**). It is as though the cells have been genetically reprogrammed, allowing them to reverse the differentiation process and to differentiate along a new and different path. This ability of differentiated cells to revert to the embryonic state and form new patterns without an intervening reproductive stage is called *totipotency*. Most living plant cells are totipotent – something akin to mammalian stem cells – and retain a complete genetic program even though not all of the information is used by the cell at any given time.

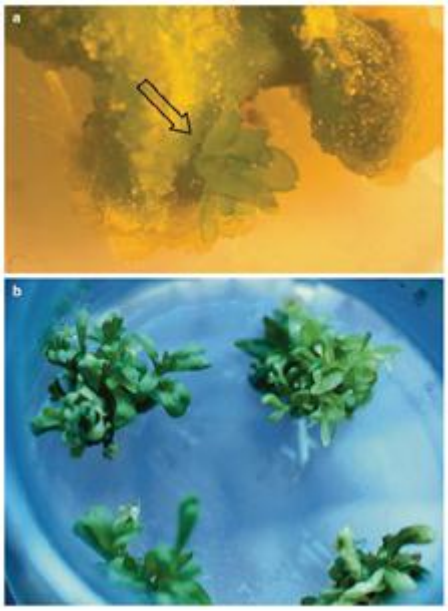


Figure 3.5 (a) Regeneration of shoots on leaf explants of carnation as a sign of totipotency. (b) Regenerated shoots can be isolated for elongation and rooting (*source: Jain S.M., Ochatt S.J., 2010*)

Development is the sum of growth and differentiation

Development is an umbrella term, referring to the sum of all of the changes that a cell, tissue, organ, or organism goes through in its life cycle. Development is most visibly manifested as changes in the form of an organ or organism, such as the transition from embryo to seedling, from a leaf primordium to a fully expanded leaf, or from the production of vegetative organs to the production of floral structures. *Embryogenesis, vegetative, and reproductive development* are the stages of sporophytic development of higher plants.

During embryogenesis, the single-celled zygote elaborates a rudimentary but polar organization that features groups of undetermined cells contained in the shoot and root apical meristems. During vegetative growth, indeterminate patterns of growth, which reflect inputs from both intrinsic programs and environmental factors, yield a variable shoot and root architecture. During reproductive development, vegetative shoot apical meristems are reprogrammed to produce a characteristic series of floral organs, including carpels and stamens, in which the haploid gametophytic generation begins.

The nature of plant meristems

Unlike animals, which are characterized by a generalized growth pattern, plant growth is limited to discrete regions where the cells retain the capacity for continued cell division. These regions are called **meristems**. Two such regions are the *apical meristems* located at the tips of roots and stems. These regions of active cell division are responsible for *primary growth*, or the increase in the length of roots and stems.

Meristems are centers of plant growth

The tip of the root is covered by a root cap, which provides mechanical protection for the meristem as the root grows through the abrasive soil medium. The root cap also secretes polysaccharides, which form a mucilaginous matrix called mucigel. Mucigel lubricates the root tip as it moves through the soil. The root cap along with its coating of mucigel is also involved in perception of gravity by roots. The *root apical meristem (RAM)* is a cluster of dividing cells located at the tip of the root just behind the root cap. Each time a cell in the meristem divides, one of the two daughter cells will be retained to continue cell division while the second daughter cell proceeds to elongate, thus increasing the length of the root and pushing the root tip through the soil. In the center of the meristem is a region of slowly dividing cells called the *quiescent zone*. Cell divisions responsible for new tissues in the elongation root and regeneration of the root cap take place around the periphery of the quiescent zone.

The *shoot apical meristem (SAM)* is structurally more complex than the root apical meristem (**Figure 3.6**). This is understandable because in addition to producing new cells that elongate and extend the length of the axis of

the shoot, the shoot apical meristern must also form primordia that give rise to lateral organs such as leaves, branches, and floral parts. Similar to the root apical meristem, each time a cell divides in the SAM, one daughter cell is left behind to elongate and move the shoot apex forward while the other daughter cell remains within the meristem to continue dividing.

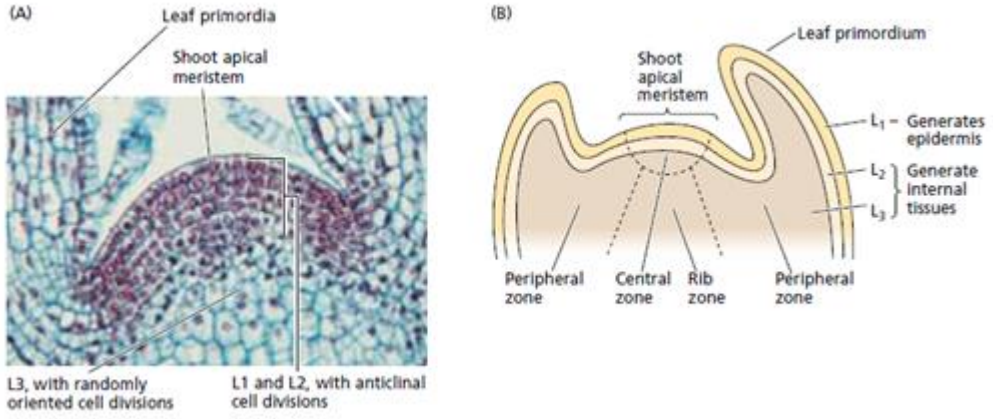


Figure 3.6 The shoot apical meristem generates the aerial organs of the plant. (A) the layered appearance of the shoot apical meristem. (B) the shoot apical meristem also has cytohistological zones (*source: Taiz L., Zeiger E., 2002*)

Tissues that are derived directly from the root and shoot apical rneristems are called *primary tissues*. The stems and roots of woody plants, however, grow in diameter as well. An increase in diameter results from the activity of a meristem called the *vascular cambium*. Tissues laid down by the vascular cambium are called *secondary tissues*, so the vascular cambium is responsible for *secondary growth*. The primary tissue of roots and shoots contains a central core of vascular, or conducting, elements. The vascular cambium develops between the xylem and phloem and produces new xylem toward the inside and new phloem toward the outside. Because of its heavy cell walls and eventual lignification, xylem is a rigid and long-lasting tissue that eventually occupies the bulk of most woody stems or trunks. Phloem is a more fragile tissue and with each year's new growth the previous year's cells tend to be pushed outward and crushed.

The root and shoot apical meristems use similar strategies to enable indeterminate growth

Although it might seem difficult to imagine two parts of a plant more different than a shoot and a root, certain features of the RAM and SAM and the roles they play in enabling indeterminate patterns of growth invite comparisons. Each of these structures features a spatially defined cluster of cells, termed initials, that are distinguished by their slow rate of division and undetermined fate. As the descendants of initials are displaced away by polarized patterns of cell division, they take on various differentiated fates that contribute to the radial and longitudinal organization of the root or shoot and to the development of lateral organs.

The development, maturation, and germination of seeds

The life of an individual plant begins when an egg nucleus in the maternal organs of a flower is fertilized by a sperm nucleus to form a zygote. Growth and differentiation of the zygote produces an *embryo* contained within a protective structure called a seed. Under appropriate conditions, the embryo within the seed will renew its growth and will continue to develop into a mature plant.

Seeds bearing embryos are formed in the flowers

Flowers appear to vary enormously in structure, yet all flowers follow the same basic plan. A generic flower consists of four whorls or circles. The two outermost whorls – the sepals and petals – are vegetative structures; and the two innermost – the *stamens and pistil* – are the male and female reproductive structures, respectively. At the base of the pistil, or female structure, is the ovary, which contains one or more ovules.

Within each ovule, a single large diploid cell, called the *megaspore mother cell*, undergoes mitosis to produce four megaspore cells. Only one megaspore cell survives and that cell undergoes meiotic division to produce an embryo sac with eight haploid nuclei. Subsequent cell division produces a mature embryo sac in which the eight

nuclei are segregated into seven cells. One of those cells is the egg. Another is the large central cell containing two polar nuclei.

The male structures, or stamens, surround the pistil and consist of an anther perched on a stalk, or filament.

In some flowers, the sepals and petals may both be colored. Pollen, containing the sperm nucleus, is produced in the anthers of the stamens. The female egg cells are produced in the ovary at the base of the pistil. Pollen is transferred to the stigma or stigmatic surface of the pistil, where it sends out a pollen tube that grows down the style and delivers the sperm nucleus to the egg.

The anther contains a large number of *microspore mother cells*, each of which undergoes meiotic division to form uninucleate, single-celled *microspores*. The microspores subsequently become encased in heavy, resistant outer walls and the nucleus divides mitotically, forming two cells – *a tube cell and a generative cell* – within the original spore wall. This is the mature *pollen grain*.

Mature pollen grains are shed from the anthers and carried to the stigmatic surface of the pistil by insects, wind, or some other vector. Once the pollen grain lands on the stigmatic surface – an event called *pollination* – the pollen grain takes up water and sends out a pollen tube that grows down the style of the pistil toward the ovule. The tube nucleus migrates down the pollen tube and appears to direct its growth. The cell wall of the generative cell breaks down and the generative nucleus divides once to form two sperm nuclei that follow the tube nucleus down the tube as it elongates.

In the final stage, the elongating pollen tube enters the ovule by growing through the *micropyle* (the space between the ends of the surrounding integuments) and releases the two sperm nuclei into the embryo sac. Ultimately, one of the two sperm nuclei enters the egg cell and fertilizes the egg cell nucleus to form the *zygote*. The second sperm nucleus enters the large central cell and fuses with the two polar nuclei to form a triploid *endosperm nucleus*. The endosperm nucleus will go on to form the primary nutritive tissue, or endosperm, for the developing embryo. The involvement of two sperm nuclei in this way is called *double fertilization*, a characteristic unique to the flowering plants or angiosperms.

Seed development is characterized by extensive cell divisions

The development of a seed begins with the fertilized ovule, or zygote. The early stage of seed development is characterized by extensive cell divisions that form the embryo and, in endospermic seeds, the tissues that store nutrients that will support the eventual germination of the seed and seedling development.

The first division of the zygote is usually transverse and immediately establishes polarity of the embryo. The upper cell is destined to become the embryo itself while the lower cell produces a stalk-like suspensor that anchors the embryo at the base of the embryo sac. The typical dicot seed will then pass through several recognizable stages (**Figure 3.7**). During the early stages of embryo development, cell division occurs throughout the entire cell mass but at the heart-shape stage both the shoot and root apical meristems begin to organize as centers of cell division.

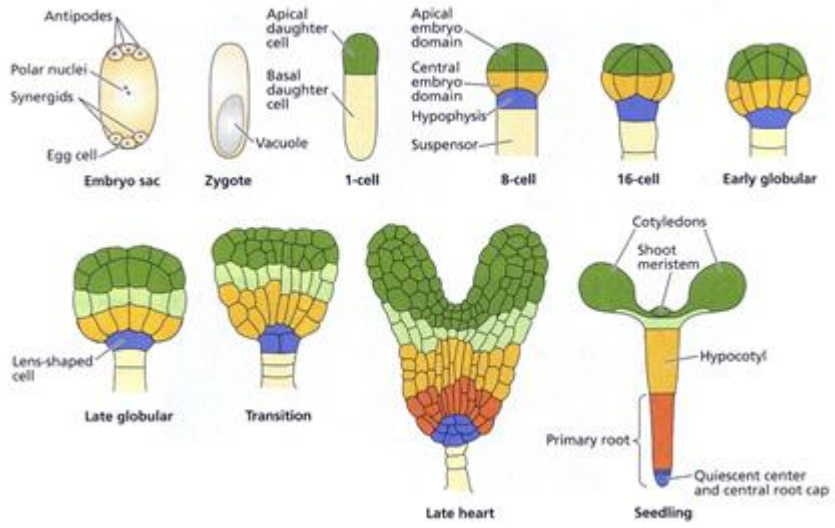


Figure 3.7 Pattern formation during Arabidopsis embryogenesis (*source: Taiz L., Zeiger E., 2010*)

Nutrients are stored in endosperm that will support germination and seedling

Throughout the development of the embryo, there is a continuous flow of nutrients from the parent plant into the endosperm or the cotyledons. In some cases, such as the cereal grains and most other monocots, he endosperm is retained until maturity and may comprise the bulk of the seed. These are called *endospermic seeds*. The endosperm of mature endospermic seeds consists of cells filled with starch along with protein and some small amounts of lipid. In some monocot seeds, the endosperm is surrounded by one or more distinctive layers of cells, called the *aleurone*. Aleurone cells are distinguished by the presence of numerous protein bodies and are the source of enzymes needed to mobilize nutrients during germination. *Endospermic dicot seeds* have retained a significant amount of endosperm and at maturity the cotyledons are thin, leaf like structures. In *nonendospermic dicot seeds* the cotyledons enlarge at the expense of the endosperm and may occupy as much as 90% of the seed volume at maturity. Both endosperm and cotyledons contain large quantities of stored carbon (in the form of carbohydrates, lipids, and protein), mineral elements, and hormones that support the growth and development of the seedling until it can establish itself as a photosynthetically competent plant.

Maturation is characterized by cessation of embryo growth and development of desiccation resistance

Maturation is terminated by a dramatic desiccation in which the water content of the seed is reduced from 80% or 90% to approximately 5%. Surrounding the mature seed is a hard coat derived from maternal tissues (the integuments) which surrounded the seed during its development in the ovary. Comprised of heavy-walled cells and covered with a thick, waxy cuticule, the seed coat often presents a significant barrier to the uptake of both water and oxygen by the seed.

Germination is resumption of embryo growth

Because seeds are severely dehydrated, any metabolic reactions take place so slowly they are scarcely detectable. Seeds are thus quiescent, or resting, organs that represent a normal hiatus in the life cycle of a plant. The embryo appears to be in a state of suspended animation, capable in some cases of surviving adverse conditions for long periods of time. Resumption of embryo growth, called *germination*, is dependent upon a number of factors, but three are especially important: *adequate water* to re-hydrate the tissues, the *presence of oxygen* to support aerobic respiration, and a *"physiological" temperature*. Although many seeds will germinate over a wide range of temperatures, the optimum range for most seeds is 25°C to 45°C.

The initial step in germination of seeds is the uptake of water and rehydration of the seed tissues by the process of *imbibition*. Like osmosis, imbibition involves the movement of water down a water potential gradient. Imbibition differs from osmosis, however, in that it does not require the presence of a differentially permeable membrane and is driven primarily by surface-acting or matric forces. In other words, imbibition involves the chemical and electrostatic attraction of water to cell walls, proteins, and other hydrophilic cellular materials. Matric potential, like osmotic potential, is always negative.

Imbibition of water is followed by a general *activation of seed metabolism* within minutes of water entering the cells, initially utilizing a few mitochondria and respiratory enzymes that had been conserved in the dehydrated state. Renewed protein synthesis is also an early event, utilizing preexisting RNA transcripts and ribosomes, as existing organelles are repaired and new organelles are formed. This is followed closely by (1) the release of hydrolytic enzymes that digest and mobilize the stored reserves, and (2) renewed cell division and cell enlargement in the embryonic axis. Seeds that store carbon reserves principally in the form of fats and oils will carry out the synthesis of hexose sugars via *gluconeogenesis*.

In most species, germination is considered complete when the radicle emerges from the seed coat. Radicle emergence occurs through a combination of cell enlargement within the radicle itself and imbibition pressures developed within the seed. Rupture of the seed coat and protrusion of the radicle allows it to make direct contact with water and nutrient salts required to support further growth of the young seedling.

Many seeds will not germinate even though the minimal environmental conditions have been met. These seeds are said to be *dormant* and will not germinate until additional conditions have been met. The most common causes of *seed dormancy* are the impermeability of the seed coat to water or oxygen or physiological immaturity of the embryo at the time the seed is shed from the mother plant. Immature seeds must undergo complex biochemical changes, collectively known as after-ripening, before they will germinate. After-ripening is usually

triggered by low temperature, a mechanism that appears to ensure that the seed will not germinate precociously in the fall but will germinate when favorable weather returns in the spring.

The pattern of development from embryo to adult

The first structure to emerge when a seed germinates is the *radicle*. The radicle, which is the nascent primary root, anchors the seed in the soil and begins the process of mining the soil for water and nutrients. As the primary roots elongates, it gives rise to branch, or lateral, roots. Unlike the situation in the shoot apical meristem, lateral roots do not originate in the root apical meristem. Lateral root primordia originate in the pericycle, a ring of meristematic cells that surround the central vascular core, or stele, of the primary root. The growing lateral root works its way through the surrounding cortex, either by mechanically forcing its way through or by secreting enzymes that digest the cortical cell walls. Lateral root primordia arise in close proximity to the newly differentiated xylem tissue, which allows vascular elements developing behind the growing tip of the lateral root to maintain connections with the xylem and phloem of the primary root.

Emergence of the radicle is followed by elongation of the *shoot axis*. It proceeds through a combination of cell division and enlargement of the cells laid down by the meristem. The rate and extent of elongation is subject to a variety of controls, including nutrition, hormones, and environmental factors such as light and temperature. The final height of a shoot is determined by the rate and extent to which internodes – the sections of stem between leaf nodes – elongate. In some plants, such as pea (*Pisum sativum*), elongation occurs primarily near the apical end of the youngest internode. The older internodes effectively complete their elongation before the next internode begins. In other plants, elongation may be spread through several internodes, which elongate and mature more or less simultaneously. Still others exhibit changing rates of elongation with successive internodes, usually increasing. In some plants, internodes fail to elongate, thus giving rise to the rosette habit in which all the leaves appear to originate from more or less the same point on the stem. This rosette habit is common in biennial plants (those that flower in the second year) such as cabbage and root crops such as carrot (*Daucus carota*) before they reach the flowering stage. Failure of internode elongation is commonly related to low levels of the plant hormone, gibberellin, since application of the hormone usually stimulates internode elongation in rosette plants.

Senescence and programmed cell death

The final stage in the development of cells, tissues, and organs is *senescence*, an aging process characterized by increased respiration, declining photosynthesis, and an orderly disassembly of macromolecules. Senescing cells and tissues are metabolically very active – a number of metabolic pathways are turned off and new pathways, principally catabolic in character, are activated. Catabolism of proteins, for example, releases organic nitrogen and sulfur in the form of soluble amines, while nucleic acids release inorganic phosphate. Chlorophyll is broken down and lipids are converted to soluble sugars via gluconeogenesis. The products of these pathways are all small, soluble molecules that are readily exported from the senescing tissue. Senescence thus enables the plant to recover nutrients from cells or tissue that have reached the end of their useful life and reallocate them to other parts of the plant that survive or for storage in the roots.

Programmed cell death (PCD) is a specialized type of senescence

Programmed cell death (PCD) is broadly defined as a process in which the organism exerts a measure of genetic control over the death of cells. PCD requires energy and is normally regulated by a distinct set of genes.

PCD is essential for normal vegetative and reproductive development. One example is the development of xylem tracheary elements. In order to function efficiently as a conduit for water transport, the protoplast of the developing tracheary element must die and be removed at maturity. PCD also operates in the formation of aerenchyma, a loose parenchymal tissue with large air spaces. Aerenchyma normally forms in the stems and roots of water lilies and other aquatic plants. These air spaces, created by a cell death program, provide channels for oxygen transport to the submerged portions of the plant. Even corn (*Zea mays*) and other terrestrial plants can be induced to form aerenchyma when subject to flooding.

PCD is also an important factor in plant responses to invading pathogens and abiotic stress. When a plant recognizes a pathogen, for example, host cells in the immediate area of the infection undergo PCD. This deprives the invading pathogen of living tissue and either slows or prevents it spread.

3. Regulation of plant growth and development

The plant cells are able to sense and respond to a wide range of *external and internal* signals. The main external signals are *light and temperature*. The effect of different temperature rates are discussed in chapters dealing with the whole plant water relationship. Also, as it is part of the stress physiology, a detailed discussion will be given later. *Intracellular regulation* of growth and development is connected mainly to the plant genetical studies. *Extracellular factors* of plant morphogenesis are *plant growth regulators* (PGRs), which often called as plant hormones or phytohormones.

3.1. Environmental factors

The effect of light in a plant's life cycle

The regulation of plant development by light, or *photomorphogenesis*, is a central theme in plant development. In order to acquire and interpret the information that is provided by light, plants have developed sophisticated photosensory systems comprised of light-sensitive *photoreceptors and signal transduction pathways*. A photoreceptor "reads" the information contained in the light by selectively absorbing different wavelengths of light. Absorption of light normally induces a conformational change in the pigment or an associated protein. Whatever the nature of the primary event, absorption of light by the photoreceptor sets into motion a cascade of events that ultimately results in a developmental response.

There are four classes of photoreceptors in plants. The **phytochromes** absorb red (R) and far-red (FR) light (ca. 660 and 735 nm, respectively) and have a role in virtually every stage of development from seed to germination to flowering. **Chryptochromes and phototropin** detect both blue (400-450 nm) and UV-A light (320-440 nm). The chryptochromes appear to play major roles during seedling development, flowering, and resetting the biological clock. Phototropin mediates phototropic responses, or differential growth in a light gradient. A fourth class of photoreceptors that mediate responses to low levels of UV-B (280-320 nm) light have not yet been characterized.

Characteristics of phytochrome-induced responses

A key breakthrough in the history of phytochrome was the discovery that the effects of red light (650–680 nm) on morphogenesis could be reversed by a subsequent irradiation with light of longer wavelengths (710–740 nm), called far-red light. This phenomenon was first demonstrated in germinating seeds, but was also observed in relation to stem and leaf growth, as well as floral induction.

The initial observation was that the germination of lettuce seeds is stimulated by red light and inhibited by far-red light. But the real breakthrough was made many years later when lettuce seeds were exposed to alternating treatments of red and far-red light. Nearly 100% of the seeds that received red light as the final treatment germinated; in seeds that received far-red light as the final treatment, however, germination was strongly inhibited (**Figure 3.8**).

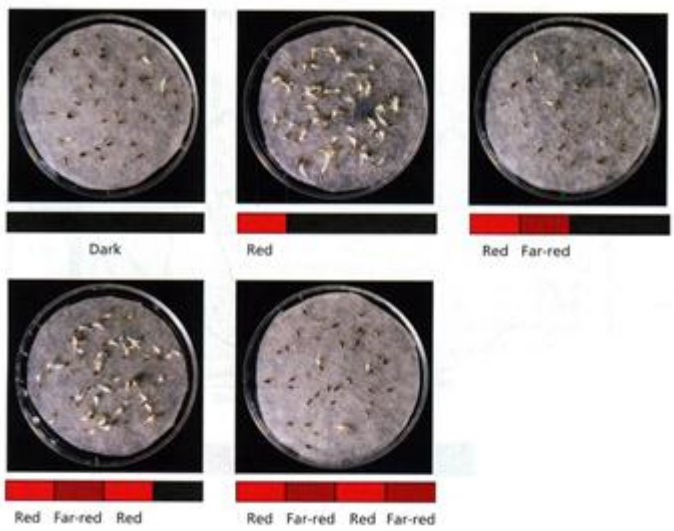


Figure 3.8 Lettuce seed germination is a typical photoreversible response controlled by phytochrome (*source: Taiz L., Zeiger E., 2010*)

Two interpretations of these results were possible. One is that there are two pigments, a red light–absorbing pigment and a far-red light–absorbing pigment, and the two pigments act antagonistically in the regulation of seed germination. Alternatively, there might be a single pigment that can exist in two interconvertible forms: a red light–absorbing form and a far-red light–absorbing form.

In dark-grown or etiolated plants, phytochrome is present in a red light-absorbing form, referred to as *Pr* because it is synthesized in this form. Pr, which to the human eye is blue, is converted by red light to a far-red light-absorbing form called *Pfr*, which is blue-green. Pfr, in turn, can be converted back to Pr by far-red light. Known as *photoreversibility*, this conversion/reconversion property is the most distinctive property of phytochrome. The interconversion of the Pr and Pfr forms can be measured *in vivo or in vitro*.

Evidence such as this has led to the conclusion that Pfr is the physiologically active form of phytochrome. In cases in which it has been shown that a phytochrome response is not quantitatively related to the absolute amount of Pfr, it has been proposed that the ratio between Pfr and Pr, or between Pfr and the total amount of phytochrome, determines the magnitude of the response.

Phytochrome responses can be distinguished by the amount of light required to induce them. The amount of light is referred to as the *fluence*, which is defined as the number of photons impinging on a unit surface area. The remarkable effects of vanishingly low levels of illumination are called *very low-fluence responses*, and they are *nonphotoreversible*. *Low-fluence responses* include most of the red/far-red *photoreversible* responses, such as the promotion of lettuce seed germination and the regulation of leaf movement.

Phytochrome signaling pathways

All phytochrome-regulated changes in plants begin with absorption of light by the pigment. After light absorption, the molecular properties of phytochrome are altered, probably affecting the interaction of the phytochrome protein with other cellular components that ultimately bring about changes in the growth, development, or position of an organ.

Molecular and biochemical techniques are helping to unravel the early steps in phytochrome action and the signal transduction pathways that lead to physiological or developmental responses. These responses fall into two general categories:

1. Ion fluxes, which cause relatively rapid turgor responses

2. Altered gene expression, which result in slower, long-term processes

Phytochrome can rapidy alter the properties of membranes, within seconds of a light pulse. Such rapid modulation has been measured in individual cells and has been inferred from the effects of red and far-red light on the surface potential of roots and oat coleoptiles, in which the lag between the production of Pfr and the onset measurable hyperpolarization (membrane potential changes) is 4.5 seconds. Changes in the bioelectric potential of cells imply changes in the flux of ions across the plasma membrane and suggest that some of the cytosolic responses of phytochrome are initiated at or near the plasma membrane.

Circadian rhythms

Various metabolic processes in plants, such as oxygen evolution and respiration, cycle alternately through high-activity and low-activity phases with a regular periodicity of about 24 hours. These rhythmic changes are referred to as *circadian rhythms*.

The period of a rhythm is the time that elapses between successive peaks or troughs in the cycle, and because the rhythm persists in the absence of external controlling factors, it is considered to be endogenous. The endogenous nature of circadian rhythms suggests that they are governed by an internal pacemaker, called an *oscillator*. The endogenous oscillator is coupled to a variety of physiological processes. An important feature of the oscillator is that it is unaffected by temperature, which enables the clock to function normally under a wide variety of seasonal and climatic conditions. The clock is said to exhibit temperature compensation.

Light is a strong modulator of rhythms in both plants and animals. Although circadian rhythms that persist under controlled laboratory conditions usually have periods one or more hours longer or shorter than 24 hours, in nature their periods tend to be uniformly closer to 24 hours because of the synchronizing effects of light at daybreak, referred to as entrainment. Both red and blue light are effective in *entrainment*. The red-light effect is

photoreversible by far-red light, indicative of phytochrome; the blue-light effect is mediated by blue-light photoreceptor(s).

Phytochrome enables plants to adapt to changing light conditions

The presence of a red/far-red reversible pigment in all green plants, from algae to dicots, suggests that these wavelengths of light provide information that helps plants adjust to their environment.

Compared with direct daylight, there is relatively more far-red light during sunset, under 5 mm of soil, or under the canopy of other plants (as on the floor of a forest). The canopy phenomenon results from the fact that green leaves absorb red light because of their high chlorophyll content but are relatively transparent to far-red light.

An important function of phytochrome is that it enables plants to sense shading by other plants. Plants that increase stem extension in response to shading are said to exhibit a shade avoidance response. As shading increases, the R:FR ratio decreases. The greater proportion of far-red light converts more Pfr to Pr, and the ratio of Pfr to total phytochrome (Pfr/Ptotal) decreases. When simulated natural radiation was used to vary the farred content, it was found that for so-called sun plants (plants that normally grow in an open-field habitat), the higher the far-red content (i.e., the lower the Pfr:Ptotal ratio), the higher the rate of stem extension.

For a “sun plant” or “shade-avoiding plant” there is a clear adaptive value in allocating its resources toward more rapid extension growth when it is shaded by another plant. In this way it can enhance its chances of growing above the canopy and acquiring a greater share of unfiltered, photosynthetically active light. The price for favoring internode elongation is usually reduced leaf area and reduced branching, but at least in the short run this adaptation to canopy shade seems to work.

The responses to blue light signals are distinct from phytochrome responses

Plants utilize light as a source of energy and as a signal that provides information about their environment. A large family of blue-light responses is used to sense light quantity and direction. These blue-light signals are transduced into electrical, metabolic, and genetic processes that allow plants to alter growth, development, and function in order to acclimate to changing environmental conditions. Blue light responses include phototropism, stomatal movements, inhibition of stem elongation, gene activation, pigment biosynthesis, tracking of the sun by leaves, and chloroplast movements within cells.

The physiology of blue-light responses varies broadly. In phototropism, stems grow toward unilateral light sources by asymmetric growth on their shaded side. In the inhibition of stem elongation, perception of blue light depolarizes the membrane potential of elongating cells, and the rate of elongation rapidly decreases. In gene activation, blue light stimulates transcription and translation, leading to the accumulation of gene products that are required for the morphogenetic response to light.

Plants can be classified according to their photoperiodic responses

As we have seen, the circadian clock enables organisms to determine the time of day at which a particular molecular or biochemical event occurs. *Photoperiodism*, or the ability of an organism to detect day length, makes it possible for an event to occur at a particular time of year, thus allowing for a seasonal response. Circadian rhythms and photoperiodism have the common property of responding to cycles of light and darkness. Perhaps all plant photoperiodic responses utilize the same photoreceptors, with subsequent specific signal transduction pathways regulating different responses.

The classification of plants according to their photoperiodic responses is usually based on flowering, even though many other aspects of plants’ development may also be affected by day length. The two main photoperiodic response categories are short-day plants and long-day plants:

1. *Short-day plants* (SDPs) flower only in short days (qualitative SDPs), or their flowering is accelerated by short days (quantitative SDPs).synthesized during long days.

2. *Long-day plants* (LDPs) flower only in long days (qualitative LDPs), or their flowering is accelerated by long days (quantitative LDPs).

The essential distinction between long-day and short-day plants is that flowering in LDPs is promoted only when the day length exceeds a certain duration, called the *critical day length*, in every 24-hour cycle, whereas promotion of flowering in SDPs requires a day length that is less than the critical day length. The absolute value

of the critical day length varies widely among species, and only when flowering is examined for a range of day lengths can the correct photoperiodic classification be established (**Figure 3.9**).

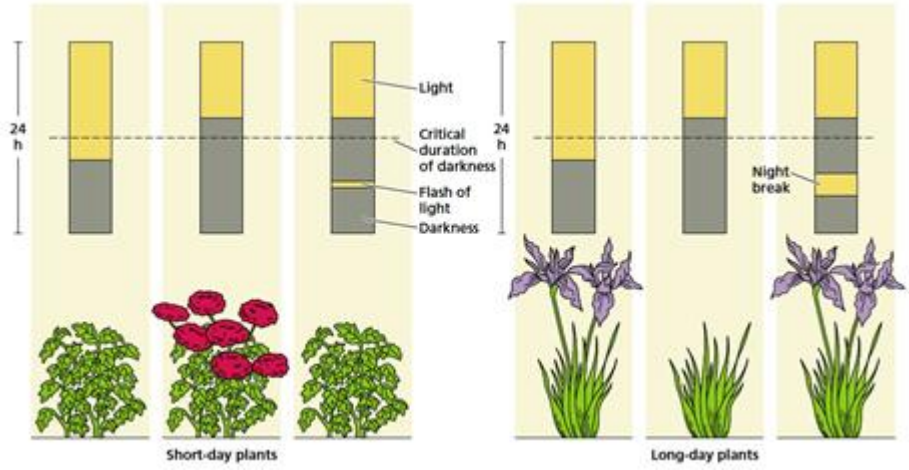


Figure 3.9 The photoperiodic regulation of flowering: effects on short-day and long-day plants (*source: Taiz L., Zeiger E., 2010*)

Long-day plants can effectively measure the lengthening days of spring or early summer and delay flowering until the critical day length is reached. Many varieties of wheat (*Triticum aestivum*) behave in this way. SDPs often flower in fall, when the days shorten below the critical day length, as in many varieties of *Chrysanthemum morifolium*. However, day length alone is an ambiguous signal because it cannot distinguish between spring and fall.

Finally, species that flower under any photoperiodic condition are referred to as day-neutral plants. *Day-neutral plants* (DNPs) are insensitive to day length. Flowering in DNPs is typically under autonomous regulation – that is, internal developmental control. Some day-neutral species, such as *Phaseolus vulgaris* (common bean) evolved near the equator where the daylength is constant throughout the year.

Vernalization: promoting flowering with cold

Plants exhibit several adaptations for avoiding the ambiguity of day length signal. One is the coupling of a temperature requirement to a photoperiodic response. Certain plant species, such as winter wheat, do not respond to photoperiod until after a *cold period (vernalization or overwintering)* has occurred.

Plants differ considerably in the age at which they become sensitive to vernalization. Winter annuals, such as the winter forms of cereals (which are sown in the fall and flower in the following summer), respond to low temperature very early in their life cycle. They can be vernalized before germination if the seeds have imbibed water and become metabolically active. Other plants, including most biennials (which grow as rosettes during the first season after sowing and flower in the following summer), must reach a minimal size before they become sensitive to low temperature for vernalization.

The effective temperature range for vernalization is from just below freezing to about 10°C, with a broad optimum usually between about 1 and 7°C. The effect of cold increases with the duration of the cold treatment until the response is saturated. The response usually requires several weeks of exposure to low temperature, but the precise duration varies widely with species and variety.

Vernalization appears to take place primarily in the shoot apical meristem. Localized cooling causes flowering when only the stem apex is chilled, and this effect appears to be largely independent of the temperature experienced by the rest of the plant. Excised shoot tips have been successfully vernalized, and where seed vernalization is possible, fragments of embryos consisting essentially of the shoot tip are sensitive to low temperature.

3.2. Plant hormones

Multicellular plants are complex organisms and their orderly development requires an extraordinary measure of coordination between cells. In order to coordinate their activities, cells must be able to communicate with each

other. The principal means of *intercellular communication* within plants are the hormones. Hormones are signal molecules that individually or cooperatively direct the development of individual cells or carry information between cells and thus coordinate growth and development.

The hormone concept in plants

The concept of hormones, the chemical messengers that enable cells to communicate with one another, arose in the study of mammalian physiology. The latter half of the nineteenth century witnessed exciting advances in physiology and medicine. The German botanist Julius Sachs (ca. 1860) postulated specific organ-forming substances in plants. He postulated that root-forming substances, for example, produced in the leaves and migrating down the stem, would account for the initiation of roots above the wound. The real beginning of plant hormone research, however, is found in a series of simple but elegant experiments conducted by Charles Darwin. It was Darwin's observations and experiments that ultimately led F. W. Went, almost half a century later, to describe a hormonal-like substance as the causative agent when plants grew toward the light. At about the same time, H. Fitting introduced the term hormone into the plant physiology literature.

Hormones are naturally occurring, organic molecules that, at low concentration, exert a profound influence on physiological processes. In addition, hormones, as defined by animal physiologists, are (1) *synthesized in a discrete organ or tissue*, and (2) *transported in the bloodstream to a specific target tissue* where they (3) *control a physiological response in a concentration-dependent manner*. While there are many parallels between animal and plant hormones, there are also some significant differences. Like animal hormones, plant hormones are naturally occurring organic substances that profoundly influence physiological processes at low concentration. The site of synthesis and mode of transport for plant hormones, however, is not always so clearly localized. Although some tissues or parts of tissues may be characterized by higher hormone levels than others, synthesis of plant hormones appears to be much more diffuse and cannot always be localized to discrete organs.

3.3. Auxins

The discovery of auxin: the first plant growth hormone

Plant hormones have been the subject of intensive investigation since auxin was first discovered almost a century ago. Darwin developed an interest in certain aspects of plant physiology. Some of these studies were summarized in the book “The Power of Movement in Plants”, co-authored by his son, Francis. One of several “movements” studied by the Darwins was the tendency of canary grass (*Phalaris canariensis*) seedlings to bend toward the light coming from a window, a phenomenon we now know as *phototropism*.

Following the publication of Darwin's book, a number of scientists confirmed and extended their observations. In 1910, Boysen-Jensen demonstrated that the stimulus would pass through an agar block and was therefore chemical in nature. In 1918, Paál showed that if the apex were removed and replaced asymmetrically, curvature would occur even in darkness (**Figure 3.10**). The active substance was first successfully isolated in 1928 by F. W. Went, then a graduate student working in his father's laboratory in Holland. Following up on the earlier work of Boysen-Jensen and Paál, Went removed the apex of oat (*Avena sativa*) coleoptiles and stood the apical pieces on small blocks of agar. Allowing a period of time for the substance to diffuse from the tissue into the agar block, he then placed each agar block asymmetrically on a freshly decapitated coleoptile. The substance then diffused from the block into the coleoptile, preferentially stimulating elongation of the cells on the side of the coleoptile below the agar block. Curvature of the coleoptile was due to differential cell elongation on the two sides. Moreover, the curvature proved to be proportional to the amount of active substance in the agar. Went's work was particularly significant in two respects: first, he confirmed the existence of regulatory substances in the coleoptile apex, and second, he developed a means for isolation and quantitative analysis of the active substance. Because Went used coleoptiles from *Avena* seedlings, his quantitative test became known as the *Avena curvature test.* Substances active in this test were called auxin, from the Greek auxein (to increase).

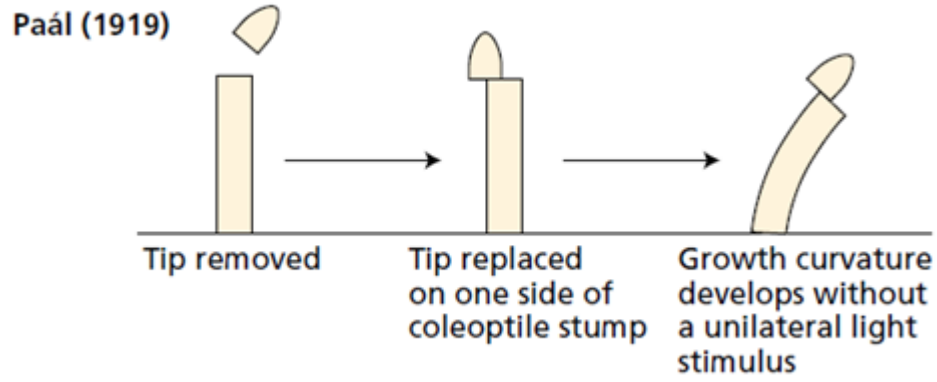
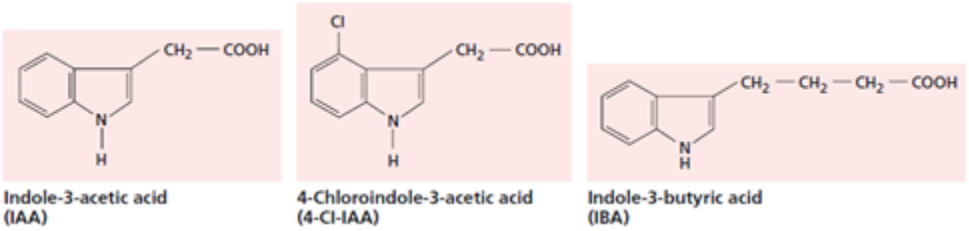


Figure 3.10 The growth promoting stimulus has chemical in nature (*source: Taiz L., Zeiger E., 2010*)

Chemical structure and biosynthesis of auxin

The pricipal natural auxin is indole-3-acetic acid

Although a large number of compounds have been discovered with auxin activity, **indole-3-acetic acid (IAA)** is the most widely distributed natural auxin. Several other auxins in higher plants were discovered later, but IAA is by far the most abundant and physiologically important. Because the structure of IAA is relatively simple (**Figure 3.11**), academic and industrial laboratories were quickly able to synthesize a wide array of molecules with auxin activity. Some of these compounds are now used widely as herbicides in horticulture and agriculture. Although they are chemically diverse, a common feature of all active auxins is a molecular distance of about 0.5 nm between a fractional positive charge on the aromatic ring and a negatively charged carboxyl group.



F**igure 3.11** Sructures of naturally occuring auxins (*source: Taiz L., Zeiger E., 2010*)

IAA is synthesized in meristems, young leaves, and developing fruits and seeds

IAA biosynthesis is associated with rapidly dividing and growing tissues, especially in shoots. Although virtually all plant tissues appear to be capable of producing low levels of IAA, shoot apical meristems and young leaves are the primary sites of auxin synthesis. Root apical meristems are also important sites of auxin synthesis, especially as the roots elongate and mature, although the root remains dependent on the shoot for much of its auxin. Young fruits and seeds contain high levels of auxin, but it is unclear whether this auxin is newly synthesized or transported from maternal tissues during development.

Multiple pathways exist for the biosynthesis of IAA

IAA is structurally related to the amino acid tryptophan, and to the tryptophan precursor indole-3-glycerol phosphate, both of which can serve as precursors for IAA biosynthesis. Molecular genetic and radioisotope labeling studies have been used to identify the enzymes and intermediate molecules involved in tryptophan-dependent IAA biosynthesis, and the order in which they function. Multiple biosynthetic pathways using tryptophan as a precursor have been shown to produce IAA in plants, and a bacterial pathway of tryptophan-dependent IAA biosynthesis has also been identified. Auxin can be covalently bound to both high and low molecular weight compounds, particularly in seeds and storage organs such as cotyledons. IAA can be conjugated to many different low molecular weight compounds like amino acids or sugars, or to high molecular weight molecules like peptides, complex glycans (multiple sugar units), or glycoproteins. IAA is rapidly released from many, but not all, conjugates by enzymatic processes. Those conjugates that can release free auxin serve as reversible storage forms of the hormone.

IAA is degraded by multiple pathways

To be effective developmental signals, hormones must be short-lived and should not accumulate over time. Auxin catabolism ensures the degradation of active hormone when the concentration exceeds the optimal level or when the response to the hormone is complete. Like IAA biosynthesis, the enzymatic breakdown (oxidation) of IAA involves more than one pathway. On the basis of isotopic labeling and metabolite identification, two oxidative pathways are probably involved in the controlled degradation of IAA. In one pathway, the indole moiety of IAA is oxidized to form oxindole-3-acetic acid (OxIAA) and subsequently, OxIAA-glucose (OxIAA-Gluc). In another pathway, IAA-aspartate conjugates are oxidized to OxIAA.

Auxin transport

The main axes of shoots and roots, along with their branches, exhibit apex-base structural polarity, and this structural polarity is dependent on the polarity of auxin transport. Soon after Went developed the coleoptile curvature test for auxin, it was discovered that IAA moves mainly from the apical to the basal end (*basipetally*) in excised oat coleoptile sections. This type of unidirectional transport is termed **polar transport.** Auxin is the only plant growth hormone that has been clearly shown to be transported polarly, and polar transport of this hormone is found in almost all plants.

Because the shoot apex serves as the primary source of auxin in the plant, polar transport has long been believed to be the principal cause of an auxin gradient extending from the shoot tip to the root tip. The major sites of polar auxin transport in the stems, leaves, and roots of most plants are the vascular parenchyma tissues, most likely those associated with the xylem. In grass coleoptiles, basipetal polar transport may also occur in nonvascular parenchyma tissues. Embryonic polar auxin transport is initially described as entirely basipetal, as the embryo has no root. The downward direction of auxin transport in the embryonic vascular parenchyma is maintained in the root vascular cylinder throughout the life of the plant.

A chemiosmotic model for polar auxin transport proposes that auxin uptake is driven by the proton motive force across the plasma membrane, while auxin efflux is driven by the membrane potential (**Figure 3.12**). The first step in polar transport is auxin influx. Auxin enters plant cells nondirectionally via passive diffusion of the protonated form (IAAH) across the phospholipid bilayer or via secondary active transport of the dissociated form (IAA-) through a 2H+-IAA-symporter. Once IAA enters the cytosol, which has a pH of approximately 7.2, nearly all of it dissociates to the anionic form. Because the membrane is impermeable to the anion, auxin accumulates inside the cell or along membrane surfaces unless it is exported by transport proteins on the plasma membrane. According to the chemiosmotic model, transport of IAA- out of the cell is driven by the negative membrane potential inside the cell.

Several compounds have been synthesized that can act as auxin transport inhibitors, including NPA (l-N-naphthylphthalamic acid), TIBA (2,3,5-triiodobenzoic acid), CPD (2-carboxyphenyl-3-phenylpropane-l,3-dione), NOA (l-napthoxyacetic acid), 2-[4-(diethylamino) -Z-hydroxybenzoyl] benzoic acid, and gravacin. NPA, TIBA, CPD, and gravacin are auxin efflux inhibitors (AEIs), while NOA is an auxin influx inhibitor. Some AEIs, such as TIBA, have weak auxin activity and inhibit polar transport in part by competing with auxin at the efflux carrier site. Other AEIs, such as CPD, NPA, and gravacin interfere with auxin transport by binding to a regulatory site. Some natural compounds, primarily flavonoids, also function as auxin efflux inhibitors.

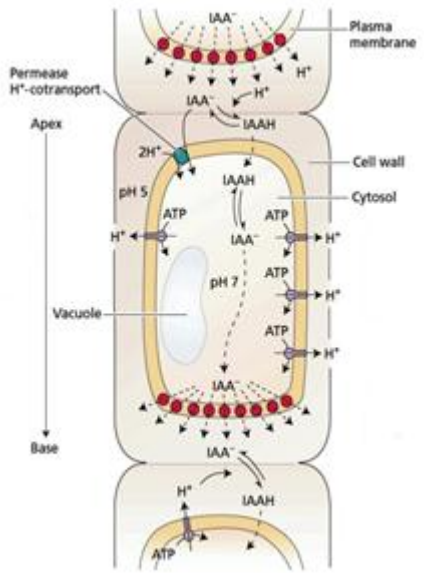


Figure 3.12 The chemiosmotic model for polar auxin transport (*source: Taiz L., Zeiger E., 2010*)

Auxin signal transduction pathway

The ultimate goal of research on the molecular mechanism of hormone action is to reconstruct each step in the signal transduction pathway, from receptor binding to the physiological response. In the case of auxin, this would seem to be a particularly daunting task because auxin affects so many physiological and developmental processes. However, the initial steps in auxin signaling are surprisingly simple, and involve binding to a small group of receptor-enzyme complexes that regulate protein degradation via the ubiquitin-proteasome pathway. Upon activation by auxin, the receptor-enzyme complex targets specific transcriptional repressors for proteolysis, resulting in the activation and derepression of auxin-responsive genes. While this mechanism appears to account for most auxin responses, a different type of auxin receptor protein may function in nontranscriptional activation and mobilization of plasma membrane H+-ATPases to cause rapid cell wall acidification and cell elongation.

Effects of auxin on growth and development

Although originally discovered in relation to growth, auxin influences nearly every stage of a plant's life cycle from germination to senescence. The morphology of a plant depends on the directed movement of auxin via the polar transport system, which maintains both basic shoot-root polarity and polarized outgrowth throughout development.

Auxins promote growth in stems and coleoptiles, while inhibiting growth in roots

The steady supply of auxin arriving at the subapical region of the stem or coleoptile is required for the continued elongation of these cells. Because the level of endogenous auxin in the elongation region of a normal healthy plant is nearly optimal for growth, spraying the plant with exogenous auxin causes only a modest and short-lived stimulation in growth. Such spraying may even be inhibitory in the case of dark-grown seedlings, which are more sensitive to supraoptimal auxin concentrations than light-grown plants.

In long-term experiments, auxin treatment of excised sections of coleoptiles or dicot stems stimulates the rate of elongation of the section for up to 20 hours. The optimal auxin concentration for elongation growth in pea stems and oat coleoptiles is typically 10-6 to 10-5 M. The inhibition observed when auxin concentrations exceed optimal levels is attributed mainly to auxin-induced ethylene biosynthesis.

Auxin control of root elongation has been more difficult to demonstrate, perhaps because auxin induces the production of ethylene, which also inhibits root growth. Recent evidence shows that these two hormones interact differentially in root tissue to control growth. However, even if ethylene biosynthesis is specifically blocked, low concentrations (10-10 to 10-9 M) of auxin promote the growth of intact roots, whereas higher concentrations (10-6 M) inhibit growth. Thus, while roots may require a minimum concentration of auxin to

grow, root growth is strongly inhibited by auxin concentrations that promote elongation in stems and coleoptiles.

The minimum lag time for auxin-induced elongation is ten minutes

When a stem or coleoptile section is excised and inserted into a sensitive growth-measuring device, the growth response to auxin can be monitored at very high resolution. Without auxin in the medium, the growth rate declines rapidly. Addition of auxin markedly stimulates the growth rate after a lag period of only 10 to 12 minutes. Beyond the optimum concentration, auxin becomes inhibitory. Both oat (*Avena sativa*) coleoptiles and soybean (*Glycine max*) hypocotyls (dicot stems) reach a maximum growth rate after 30 to 60 minutes of auxin treatment. This maximum represents a five to tenfold increase over the basal rate. The stimulation of growth by auxin requires energy, and metabolic inhibitors inhibit the response within minutes

Auxin induced proton extrusion increases cell extension

According to the widely accepted **acid growth hypothesis**, hydrogen ions act as the intermediate between auxin and cell wall loosening (**Figure 3.13**). The source of the hydrogen ions is the plasma membrane H+-ATPase, whose activity is thought to increase in response to auxin. Auxin should increase the rate of proton extrusion (wall acidification), and the kinetics of proton extrusion should closely match those of auxin-induced growth. Cell walls should contain a "wall-loosening factor" with an acidic pH optimum.

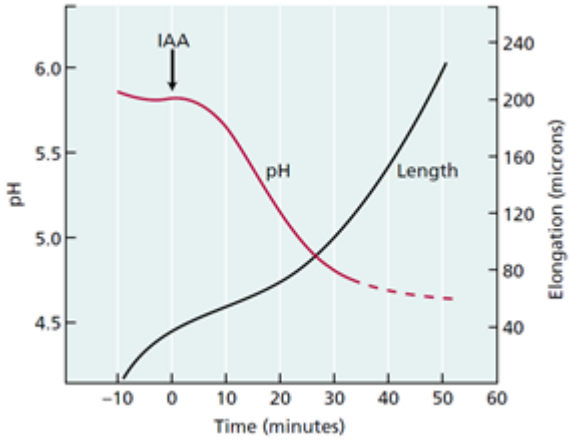


Figure 3.13 Kinetics of auxin-induced elongation and cell wall acidification in maize coleoptiles (*source: Taiz L., Zeiger E., 2010*)

Phototropism is mediated by the lateral redistribution of auxin

Phototropism, or growth with respect to light, is expressed in all shoots and some roots; it ensures that leaves will receive optimal sunlight for photosynthesis. When a shoot is growing vertically, auxin is transported polarly from the growing tip to the elongation zone. The polarity of auxin transport from tip to base is developmentally determined and is independent of orientation with respect to gravity. However, auxin can also be transported laterally, and this lateral movement of auxin lies at the heart of a model for tropisms originally proposed independently in the 1920s by two plant physiologists: Nicolai Cholodny in Russia and Frits Went in the Netherlands. According to the **Cholodny-Went model of phototropism**, the tips of grass coleoptiles are sites of high auxin concentration and have two other specialized functions: (1) the perception of a unilateral light stimulus, and (2) decrease in basipetal IAA transport and diversion to lateral transport in response to the phototropic stimulus. Thus, in response to a directional light stimulus, the auxin produced at the tip, instead of being transported basipetally, is transported laterally toward the shaded side.

Gravitropism involves lateral redistribution of auxin

Gravitropism, growth in response to gravity, enables roots to grow downward into the soil and shoots to grow upward away from the soil, responses that are especially critical during the early stages of germination. When dark-grown *Avena* seedlings are oriented horizontally, the coleoptiles bend upward in response to gravity. According to the Cholodny-Went model, auxin in a horizontally oriented coleoptile tip is transported laterally to the lower side, causing the lower side of the coleoptile to grow faster than the upper side. Early experimental evidence indicated that the tip of the coleoptile could perceive gravity and redistribute auxin to the lower side.

For example, if coleoptile tips are oriented horizontally, a greater amount of auxin diffuses into an agar block from the lower half than from the upper half.

Auxin regulates apical dominance

In most higher plants, the growing apical bud inhibits the growth of lateral (axillary) buds – a phenomenon called **apical dominance**. Removal of the shoot apex (decapitation) results in the outgrowth of one or more of the lateral buds. Not long after the discovery of auxin, it was found that IAA could substitute for the apical bud in maintaining the inhibition of lateral buds. The classic experiment performed on kidney bean (*Phaseolus vulgaris*) plants by Kenneth Thimann and Folke Skoog in the 1920s (**Figure 3.14**).

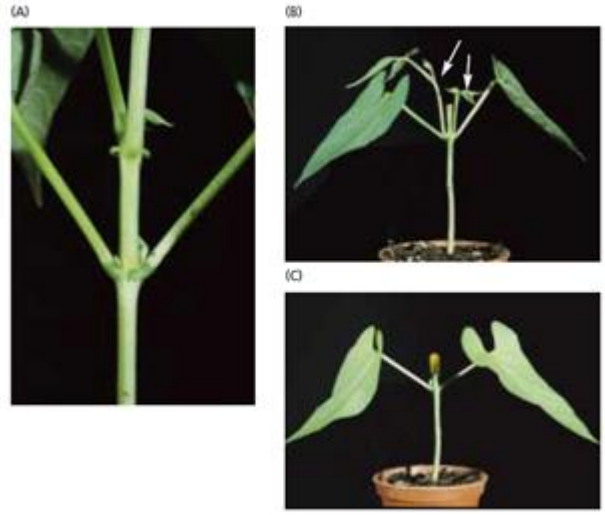


Figure 3.14 Auxin supresses the growth of axillary buds in bean plants (*source: Taiz L., Zeiger E., 2002*)

This result was soon confirmed for numerous other plant species, leading to the hypothesis that the outgrowth of the axillary bud is inhibited by auxin that is transported basipetally from the terminal bud. This hypothesis was supported by experiments that showed that a ring of the auxin transport inhibitor TIBA in lanolin paste (as a carrier) placed below the shoot apex releases the axillary buds from inhibition. Measurements of auxin levels in axillary buds have shown that following decapitation, the auxin content of the buds actually increases. In addition, application of auxin directly to the terminal bud raises the auxin concentration in the shoot but fails to inhibit normal axillary bud outgrowth. Finally, experiments with radiolabeled auxin have shown that the auxin applied at the terminal bud does not enter apical buds.

Auxin promotes the formation of lateral and adventitious roots

Although elongation of the primary root is inhibited by auxin concentrations greater than 10-8 M, initiation of lateral (branch) roots and adventitious roots is stimulated by high auxin levels. Lateral roots are commonly found above the elongation and root hair zone and originate from small groups of cells in the pericycle. Auxin stimulates these pericycle cells to divide. The dividing cells gradually form into a root apex, and the lateral root grows through the root cortex and epidermis.

Increased auxin levels or application of auxin can promote the formation of adventitious roots (roots originating from nonroot tissue). Adventitious roots arise from differentiated cells that begin to divide and develop into a root apical meristem in a manner somewhat analogous to the formation of a lateral root primordium. In horticulture, the stimulatory effect of auxin on the formation of adventitious roots has been very useful for the vegetative propagation of plants by cuttings.

Auxin delays the onset of leaf abscision

The shedding of leaves, flowers, and fruits from the living plant is known as **abscission**. These parts abscise in a region called the *abscission zone*, which, in the case of leaves, is located near the base of the petiole. In most plants, leaf abscission is preceded by the differentiation of a distinct layer of cells, the *abscission layer*, within the abscission zone. During leaf senescence, the walls of the cells in the abscission layer are digested, which causes them to become soft and weak. The leaf eventually breaks off at the abscission layer as a result of stress on the weakened cell walls.

Auxin levels are high in young leaves, progressively decrease in maturing leaves, and are relatively low in senescing leaves when the abscission process begins. The role of auxin in leaf abscission can be readily demonstrated by excision of the blade from a mature leaf, leaving the petiole intact on the stem. Whereas removal of the leaf blade accelerates the formation of the abscission layer in the petiole, application of IAA in lanolin paste to the cut surface of the petiole prevents the formation of the abscission layer. However ethylene also plays a crucial role in the regulation of abscission.

Auxin promotes fruit development

Much evidence suggests that auxin is involved in the regulation of fruit development. Auxin is produced or mobilized from storage in pollen, and the initial stimulus for fruit growth may result from pollination. Successful pollination initiates ovule growth, which is known as *fruit set*. After fertilization, fruit growth may depend on auxin from developing seeds. The endosperm may contribute auxin during the initial stage of fruit growth, and the developing embryo may take over as the main auxin source during the later stages.

3.4. Gibberellins

Gibberellins (**GAs**) are best known for their promotion of stem elongation, and GA-deficient mutants that have dwarf phenotypes have been isolated. Mendel's tall/dwarf alleles in peas are a famous example of a single gene locus that can control the level of bioactive GA and hence stem length. Such mutants have been useful in elucidating the complex pathways of GA biosynthesis, and in determining which of the GAs in a plant has intrinsic biological activity.

Although gibberellins did not become known to American and British scientists until the 1950s, they had been discovered much earlier by Japanese scientists. Rice farmers in Asia had long known of a disease that makes the rice plants grow tall but eliminates seed production. In Japan this disease was called the "foolish seedling" or *bakanae* disease. Plant pathologists investigating the disease found that the tallness of these plants was induced by a chemical secreted by a fungus that had infected the tall plants. This chemical was isolated from filtrates of the cultured fungus and called gibberellin after *Gibberella fujikuroi*, the name of the fungus.

Their structure is made up from isoprenoid units, and they are synthesized via the terpenoid pathway

Terpenes are a functionally and chemically diversegroup of molecules. With nearly 15,000 structures known, terpenes are probably the largest and most diverse class of organic compounds found in plants. The terpene family includes, in addition to the GAs, the hormones abscisic acid and brassinosteroids, the carotenoid pigments (carotene and xanthophyll), sterols (e.g., ergosterol, sitosterol, cholesterol) and sterol derivatives (e.g., cardiatic glycosides), latex (the basis for natural rubber), and many essential oils that give plants their distinctive odors and flavors.

The GAs are diterpenoids that are formed from four isoprenoid units each consisting of five carbons. They possess a tetra cyclic (four-ringed) ent-gibberellane skeleton (containing 20 carbon atoms) (**Figure 3.15**). Terpenoids are compounds made up of five-carbon isoprenoid building blocks. The GAs are diterpenoids that are formed from four such isoprenoid units. The GA biosynthetic pathway can be divided into three stages, each residing in a different cellular compartment: plastid, ER, or cytosol. As more GAs from *Gibberella* and different plant sources were characterized, a scheme was adopted to number them (GA1-GAn) in chronological order of their discovery.

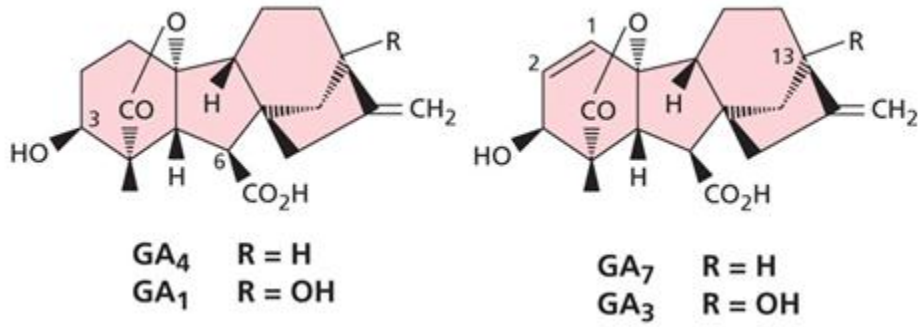


Figure 3.15 The structures of GA4, GA1, GA7, and GA3 (*source: Taiz L., Zeiger E., 2010*)

GA biosynthesis occurs in multiple plant organs, like germinating embryos, young seedlings, shoot apices, developing seeds, and even in some fungi

It is generally accepted that there are three principal sites of gibberellin biosynthesis: (1) developing seeds and fruits, (2) the young leaves of developing aical buds and elongating shoots, and (3) the apical regions of roots. Immature seeds and fruits are prominent sites of gibberellin biosynthesis. This is based on the observation that young fruits, seeds, and seed parts contain large amount of gibberellins, particularly during stages of rapid increase in size. In addition, cell-free preparations from seeds are able to actively synthesize gibberellins. The site of gibberellin biosynthesis may be developing endosperm, the young cotyledons of legumes, or the scutellum of cereal grains. As the seed matures, metabolism appears to shift in favor of gibberellin-sugar conjugates. It is not as easy to obtain clear evidence that gibberellin biosynthesis occurs in shoots and roots. This is partly because gibberellin levels are much lower in vegetative tissues. Vegetative tissues also yield cell-free preparations that are less active, suggesting that enzyme levels for gibberellin metabolism are also lower than for reproductive tissues.

The gibberellins are mobile and may act either locally or distant from their sites of synthesis

Gibberellin transport studies have been conducted largely by application of radioactively labeled GAs to either stem or coleoptile sections. Gibberellins have been detected in both phloem and xylem saps. Transport of gibberellins does not appear to be polar, as it is with auxin, but moves along with other phloem-translocated organic materials according to a source-sink relationship. Whether gibberellins are actually transported in the xylem is not clear; they could end up there simply by lateral translocation from the phloem. On the other hand, it is likely that any gibberellins synthesized in the root tip are distributed to the aerial portions of the plant through the xylem stream. It is not known whether gibberellins are transported as free hormones or in conjugated form.

Effects of gibberellins on growth and development

Though they were originally identified as the cause of disease symptoms of rice that resulted in internode elongation, endogenous GAs can influence a large number of developmental processes in addition to stem elongation. Many of these properties of GAs have been exploited in agriculture for decades, and manipulation of the GA content of crop plants affects shoot size, fruit set, and fruit growth.

Gibberellins promote seed germination via interrupting of dormancy

Many seeds, particularly those of wild plant species, do not germinate immediately after dispersal from the mother plant, and may experience a period of dormancy. Dormant seeds will not germinate even if provided with water. Abscisic acid (ABA) and bioactive GA act in an antagonistic manner, and the relative amounts of the two hormones within the seed can, in many species, determine the degree of dormancy. Light or cold treatments of dormant seeds have been shown to lower the amount of ABA and increase the concentration of bioactive GA, ending dormancy and promoting germination. Treatment of seeds with bioactive GA can often substitute for the light or cold treatment needed to break dormancy.

During germination, GAs induce the synthesis of hydrolytic enzymes, such as amylases and proteases in cereal grains (**Figure 3.16**). These enzymes degrade the stored food reserves accumulated in the endosperm or embryo as the seed matured. This degradation of carbohydrates and storage proteins provides nourishment and energy to support seedling growth.

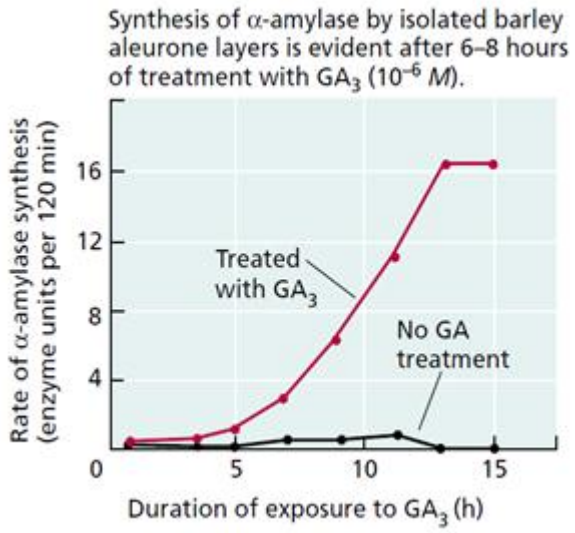


Figure 3.16 Gibberellin effect on enzyme synthesis (*source: Taiz L., Zeiger E., 2002*)

GAs can stimulate stem and root growth

GAs may not have dramatic effects on stem elongation in plants that are already "tall", since bioactive GA may not to be limiting in some tall plants. However, applied GAs can promote internode elongation very dramatically in genetically dwarf mutants, in "rosette" species, and in various members of the *Poaceae* (grass family). Exogenous GA causes such extreme stem elongation in dwarf maize plants that they resemble the tallest varieties of the same species.

Gibberellins are also important for root growth. Extreme dwarf mutants of pea and *Arabidopsis*, in which GA biosynthesis is blocked, have shorter roots than wild-type plants, and GA application to the shoot enhances both shoot and root elongation

They regulate the transition from juvenile to adult phase

Many woody perennials do not flower or produce cones until they reach a certain stage of maturity; up to that stage they are said to be juvenile. Applied GAs can regulate phase change, though whether GA hastens or retards the juvenile-to-adult transition will depend on the species. In many conifers, the juvenile phase, which may last up to 20 years, can be shortened by treatment with GA3 or with a mixture of GA4 and GA7, and much younger plants can be induced to enter the reproductive, cone-producing phase precociously.

They have influence on floral initiation and sex determination

GAs can substitute for the long-day requirement for flowering in many plants, especially rosette species. The interaction of photoperiod and GAs in flowering is complex. In plants with imperfect (unisexual) rather than perfect (hermaphroditic) flowers, sex determination is genetically regulated. However, it is also influenced by environmental factors such as photoperiod and nutritional status, and these environmental effects may be mediated by GAs. Just as in the case of the juvenile-to-adult transition, the nature of the effect of GA on sex determination can vary with species. In dicots such as cucumber (*Cucumis sativus*), hemp (*Cannabis sativa*), and spinach, GAs promote the formation of staminate (male) flowers, and inhibitors of GA biosynthesis promote the formation of pistillate (female) flowers. In some other plants, such as maize, GAs suppress stamen formation and promote pistil formation.

GAs promote pollen development and tube growth

Gibberellin-deficient dwarf mutants (e.g., in *Arabidopsis* and rice) have impaired anther development and pollen formation, and both these defects, which lead to male sterility, can be reversed by treatment with bioactive GA. In other mutants in which GA response (rather than GA biosynthesis) is blocked, the defects in anther and pollen development cannot be reversed by GA treatment, so these mutants are male-sterile. In addition, reducing the level of bioactive GA in *Arabidopsis* by overexpressing a GA deactivating enzyme severely inhibits pollen tube growth. Thus GAs seem to be required for both the development of the pollen grain and the formation of the pollen tube.

Gibberellins promote fruit set and parthenocarpy

Gibberellin application can cause fruit set (the initiation of fruit growth following pollination) and growth of some fruits. For example, stimulation of fruit set by GA has been observed in pear (*Pyrus communis*). GA-induced fruit set may occur in the absence of pollination, resulting in parthenocarpic fruit (fruit without seeds). In grape (*Vitis vinifera*), the "Thompson Seedless" variety normally produces small fruits because of early seed abortion. Fruits can be stimulated to enlarge by treatment with GA3. Both these effects of GAs on grapes are exploited commercially to produce large, seedless fruits.

They promote early seed development and germination

Some GA-deficient mutants, or transgenic plants with enhanced GA inactivation, have increased seed abortion. The failure of seeds to develop normally can be attributed to reduced levels of bioactive GAs in very young seeds. Treatment with GA will not restore normal seed development, because exogenous GA cannot enter the new seeds. However, the effect of GA deficiency on seed abortion can be negated by simultaneous expression of mutations that give a constitutive GA response. Taken together, these results provide evidence for a role for GA in the early stages of seed development.

3.5. Cytokinins

The cytokinins were discovered in the search for factors that stimulate plant cells to divide (i.e., undergo cytokinesis). A great many substances were tested in an effort to initiate and sustain the proliferation of normal stem tissues in culture. Materials ranging from yeast extract to tomato juice were found to have a positive effect, at least with some tissues. However, *in vitro* tissue culture growth was stimulated most dramatically when the liquid endosperm of coconut (also known as coconut water) was added to the culture medium.

In the 1940s and 1950s, Folke Skoog and co-workers at the University of Wisconsin tested many substances for their ability to initiate and sustain the proliferation of cultured tobacco pith tissue. They had observed that the nucleic acid base adenine had a slight promotive effect, so they tested the possibility that nucleic acids would stimulate division in this tissue. Surprisingly, aged or autoclaved herring sperm DNA had a powerful cell division-promoting effect. After much work, a small molecule was identified from the autoclaved DNA and named **kinetin**. It was shown to be an adenine (6-aminopurine) derivative, **6-furfurylaminopurine**. Kinetin is not a naturally occurring plant growth regulator, and it does not occur as a base in the DNA of any species. It is a by-product of the heat-induced degradation of DNA. Of greater importance, the discovery of kinetin suggested that naturally occurring molecules with structures similar to that of kinetin regulate cell division activity within the plant.

Cytokinins N6-substituted adenine derivatives

Naturally occurring cytokinins are all adenine derivatives with either an isoprene-related side chain or an aromatic (cyclic) side chain. The former are called *isoprenoid cytokinins* and the latter are called *aromatic cytokinins*. Although there is some variation depending on species and developmental stage, the most common isoprenoid cytokinins are **N6-(2-isopentenyl)-adenine (iP), *trans*-zeatin (tZ), and dihydrozeatin (DZ)** (**Figure 3.17**). The aromatic cytokinins, such as benzyladenine (BA) are less common and are found in only a few species.

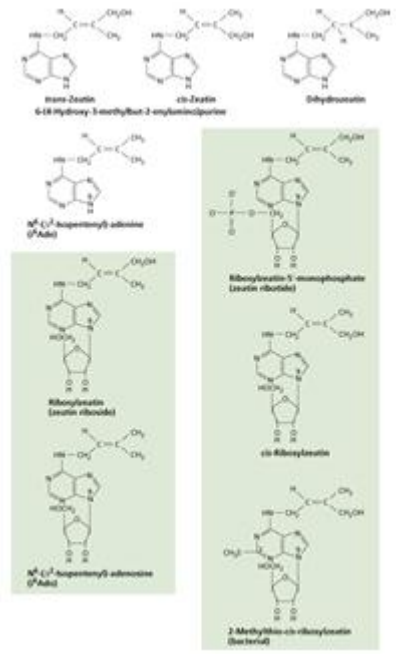


Figure 3.17 Structures of some naturally occurring cytokinins (*source: Taiz L., Zeiger E., 2010*)

Zeatin is the most abundant naturally occurring free cytokinin. Its molecular structure is similar to that of kinetin. Although they have different side chains, in both cases the side chain is linked to the nitrogen attached to C6 *(=N6)* of adenine. Because the side chain of zeatin has a double bond, it can exist in either the cis or the *trans* configuration. Since its discovery in immature maize endosperm, zeatin has been found in many plants and in some bacteria.

Cytokinin biosynthesis begins with the condensation of an isopentenyl group with the amino group of adenosine monophosphate. The reaction is catalyzed by the enzyme **adenosine phosphate-isopentenyl transferase (IPT)**. The IPT-catalyzed reaction is also the rate limiting reaction in cytokinin biosynthesis, a factor that has enabled many investigators to manipulate the cytokinin content of issues by transforming plants with genes that cause an overexpression of IPT.

Zeatin and iP are thought to be the most biologically active cytokinins in most plants. Reduction of the double bond in the side chain of zeatin would give the dihydrozeatin derivative, which is particularly active in some species of legumes.

Cytokinins are synthesized in roots, developing embryos, young leaves, fruits

A major site of cytokinin biosynthesis in higher plants is the root. High cytokinin levels have been found in roots, especially the mitotically active root tip, and in the xylem sap of roots from a variety of sources. It is generally concluded that roots are a principal source of cytokinins in most, if not all, plants and that they are transported to the aerial portion of the plant through the xylem. The delayed senescence when roots are allowed to form is apparently due to the presence of cytokinins, which are synthesized in the root and transported to the leaf through the vascular tissue.

Immature seeds and developing fruits also contain high levels of cytokinins; the first naturally occurring cytokinins were isolated from milky endosperm of maize and developing plum fruits. While there is some evidence that seeds and fruits are capable of synthesizing cytokinins, there is also evidence to the contrary. Thus it remains equally possible that developing seeds, because of their high metabolic activity and rapid growth, may simply function as a sink for cytokinins transported from the roots. On the other hand, there is now evidence that cytokinins are not always a long-distance messenger. Meristematic cells in the shoot apical meristem and floral meristems in particular are under the control of locally produced cytokinins.

Certain insects secrete cytokinins, which play a role in the formation of the galls these insects use as feeding sites. Root-knot nematodes also produce cytokinins, which may be involved in manipulating host development to produce the giant cells from which the nematode feeds.

Cytokinin receptor and signaling

In spite of the fundamental role played by cytokinins in cell division, the multiple other effects that cytokinins have on plant development have made it difficult to identify cytokinin receptors and signal chains. It has only been within the last decade, more than fifty years after Skoog and Miller purified the first cytokinin, that the first genes involved in cytokinin signaling have been identified.

The cytokinin receptor was finally discovered by T. Kakimoto and his colleagues who developed an *Arabidopsis* hypocotyl test to screen for mutants. Hypocotyl sections, or explants, respond to added cytokinins by typical cytokinin responses; rapid cell proliferation, greening, and shoot formation. The *cytokinin response 1 (cre1)* mutant shows none of these responses, even rith a tenfold increase in cytokinin concentration. This could be expected if the cytokinin receptor were either missing or nonfunctional in the mutant. Subsequent experiments confirmed that the wildtype protein CRE1 as in fact a cytokinin receptor.

CRE1 is the first component of a **two-component regulatory system** – a type of regulatory system previously known to operate in bacteria and other prokaryotes. The name comes from the bacterial configuration where **receptor** (or sensor) – the first component – activate a **response regulator (RR)** – the second component. Response regulators in turn either regulate the transcription of target genes or modulate other metabolic reactions. In addition to serving as hormone receptors, two-component regulatory systems also function in osmosensing, light sensing, and other forms of sensory perception.

Developmental and physiological effects of cytokinins

Although discovered as a cell division factor, cytokinins can stimulate or inhibit a variety of physiological, metabolic, biochemical, and developmental processes when they are applied to higher plants, and it is increasingly clear that endogenous cytokinins play an important role in the regulation of these events in the intact plant.

Cytokinins promote shoot growth by increasing cell proliferation in the shoot apical meristem

Several lines of evidence suggest that cytokinins also play key roles in the regulation of cell division *in vivo*. Much of the cell division in an adult plant occurs in the meristems. Cytokinin plays a positive role in the proliferation of cells in the shoot apical meristem. Recall that elevated levels of cytokinins may result in fasciation of shoots, a condition resulting from over-proliferation of the shoot apical meristem. Reduction of cytokinin function by reducing endogenous cytokinin levels via overexpression of cytokinin oxidase or by mutation of the IPT genes results in the opposite effect, a substantial retardation of shoot development. Disruption of cytokinin perception (e.g., in a triple-receptor mutant) also results in a reduced shoot apical meristem, leading to a stunted shoot and little or no flower production.

They inhibit root growth by promoting the exit of cells from the root apical meristem

Cytokinin plays a very different role in the root apical meristem than it does in the shoot apical meristem. In contrast to its effect on the shoot, overexpression of cytokinin oxidase in tobacco increases root growth, primarily by increasing the size of the root apical meristem. Similarly, mutations that partially disrupt cytokinin perception also cause enhanced root growth. The mechanism by which cytokinins negatively regulate root apical meristems has recently been explored. The size of a meristem is determined by the rate at which cells divide minus the rate at which cells exit the meristem by growth and differentiation. Cytokinins accelerate the process of vascular differentiation in the root tip.

Both cytokinin and auxin regulate the plant cell cycle and are needed for cell division

Cytokinins regulate cell division by affecting the controls that govern the passage of the cell through the cell division cycle. Zeatin levels peak in synchronized culture tobacco cells at the end of S phase, the G2/M phase transition, and in late G1. Inhibition of cytokinin biosynthesis blocks cell division, and application of exogenous cytokinin allows cell division to proceed. Cytokinins were discovered in relation to their ability to stimulate cell division in tissues supplied with an optimal level of auxin. Evidence suggests that both auxin and cytokinins participate in regulating the cell cycle and that they do so by controlling the activity of cyclin-dependent kinases. *Cyclin-dependent protein kinases* (CDKs), in concert with their regulatory subunits, the cyclins, are enzymes that regulate the eukaryotic cell cycle.

The auxin:cytokinin ratio regulates morphogenesis in cultured tissues

Shortly after the discovery of kinetin, it was observed that the differentiation of cultured callus tissue derived from tobacco pith segments into either roots or shoots depends on the ratio of auxin to cytokinin in the culture medium. Whereas high auxin:cytokinin ratios stimulated the formation of roots, low auxin:cytokinin ratios led to the formation of shoots. At intermediate levels, the tissue grew as an undifferentiated tissue, called *callus* (**Figure 3.18**).

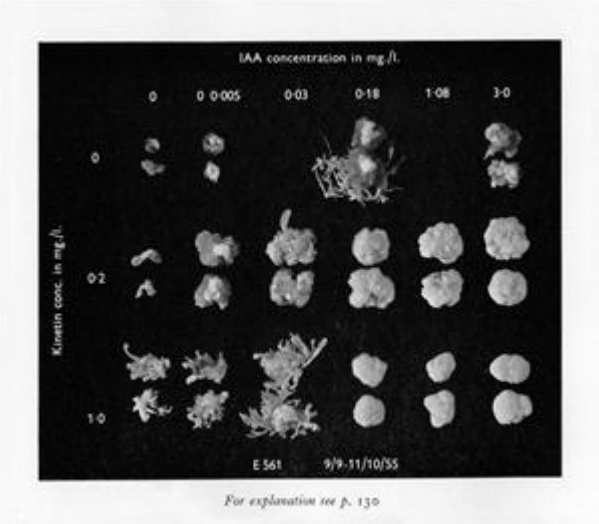


Figure 3.18 Plate 4 from Skoog and Miller (1957) showing the effect of the auxin to cytokinin ratio on the pattern of development (*source: http://www.plantphysiol.org/cgi/doi/10.1104/pp.104.900160*)

Cytokinins modify apical dominance and promote lateral bud growth

One of the primary determinants of plant form is the degree of apical dominance. Plants with strong apical dominance, such as maize, have a single growing axis with few lateral branches. In contrast, many lateral buds initiate growth in shrubby plants. Branching patterns are normally determined by light, nutrients, and genotype. Physiologically, branching is regulated by a complex interplay of hormones, including auxin, cytokinin, and a recently identified root-derived signal. Auxin transported polarly from the apical bud suppresses the growth of axillary buds. In contrast, cytokinin stimulates cell division activity and outgrowth when applied directly to the axillary buds of many species, and cytokinin-overproducing mutants tend to be bushy. In the nodal region of pea stems, auxin was found to inhibit the expression of a subset of IPT genes, which encode the enzyme catalyzing the rate-limiting step in cytokinin biosynthesis, and to elevate the expression of cytokinin oxidase, which degrades cytokinins. The combined effect of the regulation of these genes by auxin is to keep cytokinin levels low in the apical buds. Removal of the shoot apex results in a decreased auxin flow, which allows IPT levels to rise and cytokinin oxidase levels to fall. The net effect of terminal bud removal is thus an increased concentration of cytokinin in the nodal area of the stem.

Cytokinins delay leaf senescence, promote nutrient mobilization, help regulate the synthesis of photosynthetic pigments and proteins

Leaves detached from the plant slowly lose chlorophyll, RNA, lipids, and protein, even if they are kept moist and provided with minerals. This programmed aging process leading to death is termed senescence. Leaf senescence is more rapid in the dark than in the light. Treating isolated leaves of many species with cytokinins delays their senescence. Although applied cytokinins do not prevent senescence completely, their effects can be dramatic, particularly when the cytokinin is sprayed directly on the intact plant. If only one leaf is treated, it remains green after other leaves of similar developmental age have yellowed and dropped off the plant. If a small spot on a leaf is treated with cytokinin, that spot will remain green after the surrounding tissues on the same leaf begin to senesce. The cytokinins involved in delaying senescence are primarily zeatin riboside and dihydrozeatin riboside, which may be transported into the leaves from the roots through the xylem, along with the transpiration stream.

Cytokinins influence the movement of nutrients into leaves from other parts of the plant, a phenomenon known as cytokinin-induced nutrient mobilization. Thus, the nutrient status of the plant regulates cytokinin levels, and in turn the ratio of cytokinin to auxin determines the relative growth rates of roots and shoots: High cytokinin concentrations promote shoot growth, and, conversely, high auxin levels promote root growth. In the presence

of low nutrient levels, cytokinin levels are also low, resulting in an increase in root growth and allowing the plant to more effectively acquire the nutrients present in the soil. In contrast, optimal levels of soil nutrients promote increased cytokinin levels, which favor shoot growth, thus maximizing photosynthetic capacity.

If etiolated leaves are treated with cytokinin before being illuminated, they form chloroplasts with more extensive grana, and chlorophyll and photosynthetic enzy.mes are synthesized at a greater rate upon illumination. These results suggest that cytokinins – along with other factors, such as light, nutrition, and development – regulate the synthesis of photosynthetic pigments and proteins.

Cytokinin-overproducing plants have delayed senescence and yield more grain

Some of the consequences of altering cytokinin function could be highly beneficial for agriculture if synthesis of the hormone can be controlled. Because leaf senescence is delayed in the cytokinin-overproducing plants, it should be possible to extend their photosynthetic productivity. Indeed, when an ipt gene is expressed in lettuce from a senescence-inducible promoter, leaf senescence is strongly retarded, similar to the results observed in tobacco (**Figure 3.19**).

Figure 3.19 Leaf senescence is retarded in a transgenic tobacco plant containing a cytokinin biosynthesis gene, ipt (*source: Taiz L., Zeiger E., 2010*)

3.6. Ethylene

Ethylene is another class of hormones with a single representative. It is a simple gaseous hydrocarbon with the chemical structure H2C=CH2. Ethylene is apparently not required for normal vegetative growth, although it can have a significant impact on the development of roots and shoots. Ethylene appears to be synthesized primarily in response to stress and may be produced in large amounts by tissues undergoing senescence or ripening. It is commonly used to enhance ripening in bananas and other fruits that are picked green for shipment as well.

Ethylene can be produced by almost all parts of higher plants

Ethylene occurs in all plant organs – roots, stems, leaves, bulbs, tubers, fruits, seeds, and so on – although the rate of production may vary depending on the stage of development. Ethylene production will also vary from tissue to tissue within the organ, but is frequently located in peripheral tissues. In peach and avocado seeds, for example, ethylene production appears to be localized primarily in the seed coats, while in tomato fruit and mung bean hypocotyls it originates from the epidermal regions.

Ethylene production increases during leaf abscission and flower senescence, as well as during fruit ripening. Any type of wounding can induce ethylene biosynthesis, as can physiological stresses such as flooding, disease, and temperature or drought stress. In additon, infection by various pathogens can also elevate ethylene biosynthesis.

The amino acid methionine is the precursor of ethylene

M. Lieberman and L. W. Mapson first demonstrated in 1964 that methionine was rapidly converted to ethylene in a cell-free, nonenzymatic model system. In subsequent studies, Lieberman and coworkers confirmed that plant tissues such as apple fruit converted [l4C]-methionine to [14C]-ethylene and that the ethylene was derived from the third and fourth carbons of methionine. Little progress was made until 1977 when D. Adams and F. Yang demonstrated that S-adenosylmethionine (SAM) was an intermediate in the conversion of methionine to ethylene by apple tissue. In 1979, Adams and Yang further demonstrated the accumulation of *1-aminocyclopropane-1-carboxylic acid* (ACC) in apple tissue fed [13C]-methionine under anaerobic conditions – conditions that inhibit the production of ethylene. However, upon reintroduction of oxygen, the labeled ACC was rapidly converted to ethylene. ACC is a nonprotein amino acid that had been isolated from ripe apples in 1957, but its relationship to ethylene was not obvious at that time. These results established that ACC is an intermediate in the biosynthesis of ethylene.

The ethylene biosynthesis is a three-step pathway in higher plants is shown. In the first step, an adenosine group (i.e., adenine plus ribose) is donated to methionine by a molecule of ATP, thus forming SAM. An ATP requirement is consistent with earlier evidence that ethylene production is blocked by inhibitors of oxidative phosphorylation, such as 2,4-dinitrophenol. Conversion of methionine to SAM is catalyzed by the enzyme methionine adenosyltransferase or SAM synthetase.

The cleavage of SAM to yield 5'-methylthio-adenosine (MTA) and ACC, mediated by the enzyme *ACC synthase*, is the rate-limiting step. ACC synthase was the first enzyme in the pathway to be studied in detail. The enzyme has been partially purified from tomato and apple fruit but, because of its instability and low abundance, progress toward its purification and characterization has been slow. Ethylene biosynthesis is stimulated by several factors, including developmental state, environmental conditions, other plant hormones, and physical and chemical injury. Ethylene biosynthesis also varies in a circadian manner, peaking during the day and reaching a minimum at night.

The primary steps in ethylene action are likely similar: binding to a receptor, followed by activation of signal transduction pathways

Unbound ethylene receptors are negative regulators of the response pathway. In *Arabidopsis*, tomato, and probably most other plant species, the ethylene receptors are encoded by multi gene families. Targeted disruption (complete inactivation) of the five *Arabidopsis* ethylene receptors (ETRl, ETR2, ERSl, ERS2, and EIN4) has revealed that they are functionally redundant. That is, disruption of any single gene encoding one of these proteins has no effect, but a plant with disruptions in multiple receptor genes exhibits a constitutive ethylene response phenotype.

The observation that ethylene responses, such as the triple response, become constitutive when the receptors are disrupted indicates that the receptors are normally "on" (i.e., in the active state) in the absence of ethylene, and that the function of the receptor minus its ligand (ethylene), is to shut off the signaling pathway that leads to the response. Binding of ethylene "turns off" (inactivates) the receptors, thus allowing the response pathway to proceed.

Effects of ethylene on plant growth and development

Ethylene affects the transcription of numerous genes via specific transcription factors

One of the primary effects of ethylene signaling is an alteration in the expression of various target genes. Ethylene affects the mRNA transcript levels of numerous genes, including those that encode cellulase and genes related to ripening and ethylene biosynthesis. Regulatory sequences called *ethylene response elements*, or EREs, have been identified among the ethylene-regulated genes.

The hormone promotes the ripening of some fruits

In everyday usage, the term fruit ripening refers to the changes in fruit that make it ready to eat. Such changes typically include softening due to the enzymatic breakdown of the cell walls, starch hydrolysis, sugar accumulation, and the disappearance of organic acids and phenolic compounds, including tannins.

Because of their importance in agriculture, the vast majority of studies on fruit ripening have focused on edible fruits. Ethylene has long been recognized as the hormone that accelerates the ripening of edible fruits. Exposure of such fruits to ethylene hastens the processes associated with ripening, and a dramatic increase in ethylene

production accompanies the initiation of ripening. However, surveys of a wide range of fruits have shown that not all of them respond to ethylene.

All fruits that ripen in response to ethylene exhibit a characteristic respiratory rise called a *climacteric* before the ripening phase. Such fruits also show a spike of ethylene production immediately before the respiratory rise (**Figure 3.20**). Apples, bananas, avocados, and tomatoes are examples of climacteric fruits. In contrast, fruits such as citrus fruits and grapes do not exhibit the respiration and ethylene production rise and are called *nonclimacteric* fruits. In climacteric fruits, treatment with ethylene induces the fruit to produce additional ethylene, a response that can be described as autocatalytic.

Ethylene inhibits hypocotyl elongation

At concentrations above 0.1 µL L-1, ethylene changes the growth pattern of seedlings by reducing the rate of elongation and increasing lateral expansion, leading to swelling of the hypocotyl or the epicotyl. In dicots, this swelling is part of the *triple response*, which, in *Arabidopsis*, consists of inhibition of hypocotyl elongation combined with hypocotyl swelling, inhibition of root elongation, and exaggeration of the curvature of the apical hook.

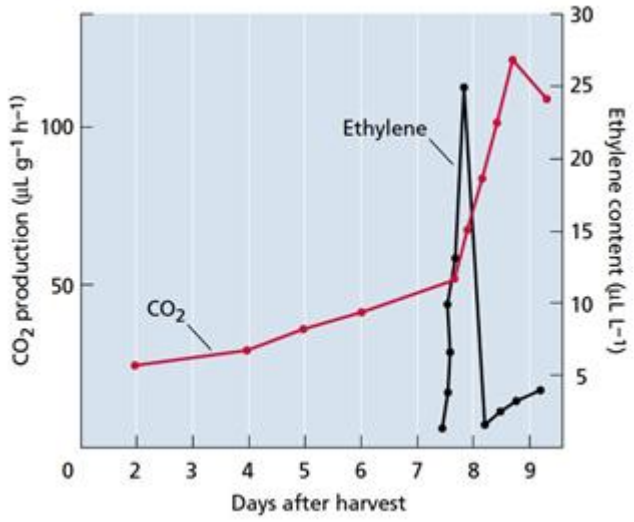


Figure 3.20 Ethylene production and respiration during banana ripening (*source: Taiz L., Zeiger E., 2010*)

It regulates flowering, sex determination, and defence responses in some species

Although ethylene inhibits flowering in many species, it induces flowering in pineapple and its relatives, and it is used commercially for synchronization of pineapple fruit set. Flowering of other species, such as mango, is also initiated by ethylene. On plants that have separate male and female flowers (monoecious species), ethylene may change the sex of developing flowers. The promotion of female flower formation in cucumber is one example of this effect. Recently, a gene responsible for andromonoecy (plants carrying both male and bisexual flowers) in melons was identified as encoding an ACC synthase. A mutation that reduces the activity of this ACC synthase gene results in the formation of the bisexual flowers in these andromonoecious lines.

Pathogen infection and disease will occur only if the interactions between host and pathogen are genetically compatible. However, ethylene production generally increases in response to pathogen attack in both compatible (i.e., pathogenic) and noncompatible (nonpathogenic) interactions. The discovery of ethylene-insensitive mutants has facilitated the assessment of the role of ethylene in the response to various pathogens. The involvement of ethylene in pathogenesis is complex and depends on the particular host-pathogen interaction. For example, blocking ethylene responsiveness does not affect the resistance responses of *Arabidopsis* to *Pseudomonas* bacteria or of tobacco to tobacco mosaic virus. In compatible interactions of these pathogens and hosts, however, elimination of ethylene responsiveness prevents the development of disease symptoms, even though the growth of the pathogen appears to be unaffected.

Ethylene is active in leaf and flower senescence and in leaf abscision

Senescence is a genetically programmed developmental process that affects all tissues of the plant. Research has provided several lines of physiological evidence that support roles for ethylene and cytokinins in the control of leaf senescence:

- exogenous applications of ethylene or ACC (the precursor of ethylene) accelerate leaf senescence, and treatment with exogenous cytokinins delays leaf senescence;
- enhanced ethylene production is associated with chlorophyll loss and color fading, which are characteristic features of leaf and flower senescence; an inverse correlation has been found between cytokinin levels in leaves and the onset of senescence;
- inhibitors of ethylene synthesis (e.g., AVG or Co2+) and action (e.g., Ag+ -STS- or CO2) retard leaf and flower senescence (**Figure 3.21**).

Taken together, these physiological studies suggest that senescence is regulated by the balance of ethylene and cytokinin. In addition, abscisic acid has been implicated in the control of leaf senescence.

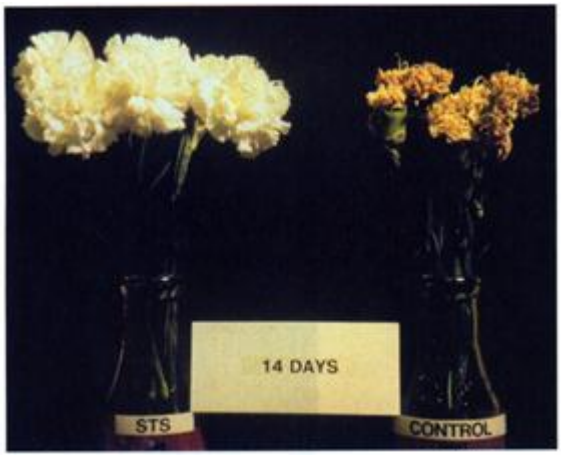


Figure 3.21 Inhibition of flower senescence by inhibition of ethylene action (*source: Taiz L., Zeiger E., 2010*)

The shedding of leaves, fruits, flowers, and other plant organs is termed abscission. Abscission takes place in specific layers of cells called abscission layers, which become morphologically and biochemically differentiated during organ development. Weakening of the cell walls at the abscission layer depends on cell wall-degrading enzymes such as cellulase and polygalacturonase. Ethylene appears to be the primary regulator of the abscission process, with auxin acting as a suppressor of the ethylene effect. However, supraoptimal auxin concentrations stimulate ethylene production, which has led to the use of auxin analogs as defoliants. Its action is based on its ability to increase ethylene biosynthesis, thereby stimulating leaf abscission.

During the *early phase of leaf maintenance*, auxin from the leaf prevents abscission by maintaining the cells of the abscission zone in an ethylene-insensitive state. It has long been known that removal of the leaf blade (the site of auxin production) promotes petiole abscission. Application of exogenous auxin to petioles from which the leaf blade has been removed delays the abscission process. However, application of auxin to the proximal side of the abscission zone (i.e., the side closest to the stem) actually accelerates the abscission process.

In the *shedding induction phase*, the amount of auxin from the leaf decreases and the ethylene level rises. Ethylene appears to decrease the activity of auxin both by reducing its synthesis and transport and by increasing its destruction. The reduction in the concentration of free auxin increases the response of specific target cells to ethylene. The shedding phase is characterized by the induction of genes encoding specific hydrolytic enzymes of cell wall polysaccharides and proteins.

The target cells, located in the *abscission zone*, synthesize cellulase and other polysaccharide-degrading enzymes, and secrete them into the cell wall. The activities of these enzymes lead to cell wall loosening, cell separation, and abscission.

3.7. Abscisic acid

Unlike auxins, gibberellins, and cytokinins, the hormone **abscisic acid (ABA)** is represented by a single 15-carbon sesquiterpene. ABA also appears to have a more limited range of specific effects than auxins, gibberellins, and cytokinins. The name is based on the once held belief that it was involved in the abscission of leaves and other organs. It now appears to have nothing to do with abscision, but the name has stuck.

The primary functions of ABA are (1) prohibiting precocious germination and promoting dormancy in seeds and (2) inducing stomatal closure and the production of molecules that protect cells against desiccation in times of water stress.

The chemical structure of ABA determines its physiological activity

Once the structure of ABA had been determined, two possible pathways for the synthesis of ABA were proposed. In the "direct pathway", ABA would be synthesized from a 15-carbon terpenoid precursor such as farnesyl diphosphate. By the late 1970s it had been clearly established that this pathway was operative in certain fungal plant pathogens that actively synthesized ABA, but not in plants themselves. According to the second, or "indirect pathway", ABA was produced from the cleavage of a carotenoid such as β-carotene. Originally proposed in the late 1960s, the indirect pathway was based on structural similarities between carotenoid pigments and ABA and has since received support from a variety of biochemical studies, 18O2-labeling experiments, and, most recently, the characterization of ABA biosynthetic mutants. The cleavage of carotenoids, especially β -carotene, to produce useful biochemicals is not without precedent. The cyanobacterium Microcystis, for example, produces a C10 metabolite by cleavage of β-carotene. Mammals produce vitamin A by cleavage of β-carotene and cleavage of β-carotene to produce 2 molecules of the photoreceptor retinal (C20) has been reported.

ABA signal transduction pathways

ABA is involved in *short-term physiological effects* (e.g., stomatal closure), as well as *long-term developmental processes* (e.g., seed maturation):

- rapid physiological responses frequently involve alterations in the fluxes of ions across membranes and usually involve regulation of certain genes as well, as evidenced by the fact that a variety of ABA-stimulated transcription factors that are expressed in guard cells regulate stomatal aperture;
- in contrast, long-term processes inevitably involve major changes in the pattern of gene expression.

Comparisons of total transcript populations have shown that at least 10% of the genes in both *Arabidopsis* and rice are regulated by ABA. Signal transduction pathways, which amplify the primary signal generated when the hormone binds to its receptor, are required for both the short-term and the long-term effects of ABA.

Developmental and physiological effects of ABA

Abscisic acid plays primary regulatory roles in the initiation and maintenance of seed and bud dormancy and in the plant's response to stress, particularly water stress. In addition, ABA influences many other aspects of plant development by interacting, usually as an antagonist, with auxin, cytokinin, gibberellin, ethylene, and brassinosteroids.

In seed development, ABA promotes the synthesis of storage proteins and lipids, as well as special proteins

The ABA content of seeds is very low early in embryogenesis, reaches a maximum at about the halfway point, and then gradually falls to low levels as the seed reaches maturity. Thus there is a broad peak of ABA accumulation in the seed corresponding to mid-to late embryogenesis. The hormonal balance of seeds is complicated by the fact that not all the tissues have the same genotype. The seed coat is derived from maternal tissues, the zygote and endosperm are derived from both parents. Genetic studies with ABA -deficient mutants of Arabidopsis have shown that the zygotic genotype controls ABA synthesis in the embryo and endosperm and is essential to dormancy induction, whereas the maternal genotype controls the major, early peak of ABA accumulation and helps suppress vivipary in mid-embryogenesis. During mid-to late embryogenesis, when seed ABA levels are highest, seeds accumulate storage compounds that will support seedling growth at germination. Another important function of ABA in the developing seed is to promote the acquisition of desiccation tolerance. As maturing seeds begin to lose water, embryos accumulate sugars and so-called late embryogenesis-abundant (LEA) proteins. Physiological and genetic studies have shown that ABA affects the synthesis of LEAs and of storage proteins and lipids.

Seed dormancy and germination are controlled by the ratio of ABA to gibberellic acid (GA)

During seed maturation, the embryo desiccates and enters a quiescent phase. Seed germination can be defined as the resumption of growth of the embryo of the mature seed. Germination depends on the same environmental conditions as vegetative growth does: water and oxygen must be available, the temperature must be suitable, and there must be no inhibitory substances present.

In many cases a viable (living) seed will not germinate even if all the necessary environmental conditions for growth are satisfied. This phenomenon is termed *seed dormancy*. Seed dormancy introduces a temporal delay in the germination process that provides additional time for seed dispersal over greater geographic distances. It also maximizes seedling survival by preventing germination under unfavorable conditions. Seed dormancy may result from *coat-imposed dormancy*, *embryo dormancy*, or both. Dormancy imposed on the embryo by the seed coat and other enclosing tissues, such as endosperm, pericarp, or extrafloral organs, is known as coat-imposed dormancy. The embryos of such seeds will germinate readily in the presence of water and oxygen once the seed coat and other surrounding tissues have been either removed or damaged. Seed dormancy that is intrinsic to the embryo and is not due to any influence of the seed coat or other surrounding tissues is called embryo dormancy.

Embryo dormancy is thought to be due to the presence of inhibitors, especially ABA, as well as the absence of growth promoters, such as GA. Maintenance of dormancy in imbibed seeds requires *de novo* ABA biosynthesis (**Figure 3.22**), and the loss of embryo dormancy is often associated with a sharp decrease in the ratio of ABA to GA. The levels of ABA and GA are regulated by their synthesis and catabolism, which are catalyzed by specific isozymes whose expression is controlled by developmental and environmental factors.

Figure 3.22 Germinating of ABA-deficient seeds in the fruit while still attached to the plant (*source: Taiz L., Zeiger E., 2010*)

In germinating seeds, ABA inhibits the GA induced synthesis of hydrolitic enzymes

In addition to the ABA-GA antagonism affecting seed dormancy, ABA inhibits the GA-induced synthesis of hydrolytic enzymes that are essential for the breakdown of storage reserves in germinating seeds. For example, GA stimulates the aleurone layer of cereal grains to produce ex-amylase and other hydrolytic enzymes that break down stored resources in the endosperm during germination. ABA inhibits this GA-dependent enzyme synthesis by inhibiting the transcription of α-amylase mRNA. ABA exerts this inhibitory effect via at least two mechanisms, one direct and one indirect:

- a protein originally identified as an activator of ABA-induced gene expression, VPl, acts as a transcriptional repressor of some GA-regulated genes,
- ABA represses the GA-induced expression of GAMYB, a transcription factor that mediates the GA induction of α-amylase expression.

ABA promotes root growth and inhibits shoot growth at low water potentials

Despite the traditional view of ABA as a growth inhibitor, endogenous ABA restricts shoot growth only under water stress conditions. Moreover, under these conditions, when ABA levels are high, endogenous ABA exerts a strong positive effect on primary root growth by suppressing ethylene production. The overall effect is a

dramatic increase in the root:shoot ratio at low water potentials, which, along with the effect of ABA on stomatal closure, helps the plant cope with water stress. Furthermore, the temporary inhibition of lateral root outgrowth promotes exploration of new areas of soil, and permits replacement of dehydrated laterals following rehydration. It is not clear how different ABA levels lead to opposite effects on growth, but these effects may reflect signaling through receptors with different functional ranges of sensitivity or different downstream signaling elements in roots versus shoots.

ABA greatly accelerates the senescence of leaves, thereby increasing ethylene formation and stimulating abscision

Abscisic acid was originally isolated as an abscission causing factor. However, it has since become evident that ABA stimulates abscission of organs in only a few species and that the hormone primarily responsible for causing abscission is ethylene. On the other hand, ABA is clearly involved in leaf senescence, and through its promotion of senescence it might indirectly increase ethylene formation and stimulate abscission. Leaf senescence has been studied extensively. Leaf segments senesce faster in darkness than in light, and they turn yellow as a result of chlorophyll breakdown. In addition, the breakdown of proteins and nucleic acids is increased by the stimulation of several hydrolases. ABA greatly accelerates the senescence of both leaf segments and attached leaves.

ABA accumulates in dormant buds, inhibiting their growth; it may interact with growth-promoting hormones

ABA was originally suggested as the dormancy-inducing hormone because it accumulates in dormant buds and decreases after the tissue is exposed to low temperatures. However, later studies showed that the ABA content of buds does not always correlate with the degree of dormancy. As we saw in the case of seed dormancy, this apparent discrepancy might reflect interactions between ABA and other hormones; perhaps bud dormancy and growth are regulated by the balance between bud growth inhibitors, such as ABA, and growth-inducing substances, such as cytokinins and gibberellins. Much progress has been achieved in elucidating the role of ABA in seed dormancy by the use of ABA-deficient mutants. However, progress on the role of ABA in bud dormancy, a characteristic of woody perennials, has lagged because of the lack of a convenient genetic system. This discrepancy illustrates the tremendous contribution that genetics and molecular biology have made to plant physiology, and underscores the need for extending such approaches to woody species.

Abscisic acid closes stomata in response to water stress

ABA accumulates in water-stressed (that is, wilted) leaves and exogenous application of ABA is a powerful inhibitor of stomatal opening. The precise role of ABA in stomatal closure in water-stressed whole plants has, however, been difficult to decipher with certainty. This is because ABA is ubiquitous, often occurring in high concentrations in nonstressed tissue. Also, some early studies indicated that stomata would begin to close before increases in ABA content could be detected.

According to current thinking, the initial detection of water stress in leaves is related to its effects on photosynthesis. Inhibition of electron transport and photophosphorylation in the chloroplasts would disrupt proton accumulation in the thylakoid lumen and lower the stroma pH. At the same time, there is an increase in the pH of the apoplast surrounding the mesophyll cells. The resulting pH gradient stimulates a release of ABA from the mesophyll cells into the apoplast, where it can be carried in the transpiration stream to the guard cells.

Stomatal closure does not always rely on the perception of water deficits and signals arising within the leaves. In some cases it appears that the stomata close in response to soil desiccation well before there is any measurable reduction of turgor in the leaf mesophyll cells. Several studies have indicated a feed-forward control system that originates in the roots and transmits information to the stomata. In these experiments, plants are grown such that the roots are equally divided between two containers of soil. Water deficits can then be introduced by withholding water from one container while the other is watered regularly. Control plants receive regular watering of both containers. Stomatal opening along with factors such as ABA levels, water potential, and turgor are compared between half-watered plants and fully watered controls. Typically, stomatal conductance, a measure of stomatal opening, declines within a few days of withholding water from the roots (**Figure 3.23**), yet there is no measurable change in water potential or loss of turgor in the leaves. Furthermore, ABA is readily translocated from roots to the leaves in the transpiration stream, even when roots are exposed to dry air. These results suggest that ABA is involved in some kind of early warning system that communicates information about soil water potential to the leaves.

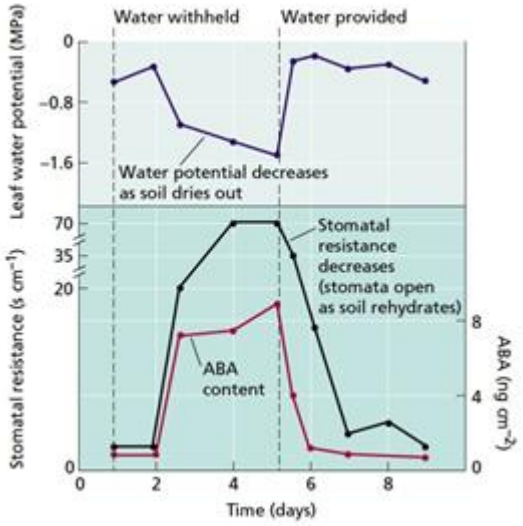


Figure 3.23 Changes in water potential, stomatal resistance, and ABA content in corn in response to water stress (*source: Taiz L., Zeiger E., 2010*)

3.8. Brassinosteroids

Brassinosteroids (BRs) are steroid hormones with a chemical structure similar to the steroid hormones in animals. Brassinosteroids elicit an impressive array of developmental responses, including an increased rate of stem and pollen tube elongation, increased rates of cell division (in the presence of auxin and cytokinin), seed germination, leaf morphogenesis, apical dominance, inhibition of root elongation, vascular differentiation, accelerated senescence, and cell death. Brassinosteroids are also implicated in mediating responses to both abiotic and biotic stress, including salt, drought, temperature extremes, and pathogens.

BRs cause dramatic changes in growth and differentiation at very low concentration

The study of brassinosteroids as plant hormones dates back to the early 1970s, when a group of agricultural researchers began screening pollen, already known as a rich source of growth-promoting substances. The result was a complex mixture of lipids that stimulated elongation of bean second internodes (**Figure 3.24**). Because the most active preparations were isolated from pollen of the rape plant (*Brassica napus*), the active substances were referred to collectively as **brassins**. Many of the effects of the brassins were similar to those of GA, leading many to believe the extracts were simply crude extracts of gibberellins, rather than a new class of hormones as originally proposed. However, in 1979, M. D. Grove and his coworkers identified the active component as **brassinolide (BL)**.

Figure 3.24 Bean second-internode bioassay for brassinosteroids (*source: Taiz L., Zeiger E., 2010*)

Brassinosteroids are synthesized from campesterol

Brassinosteroids are polyhydroxylated plant sterols – lipoidal substances related biosynthetically to the gibberellins and abscisic acid. Plants synthesize a large number and variety of sterols, including sitosterol, stigmasterol, cholesterol, and *campesterol*. Sterols are triterpenoids, C30 molecules that are derived from acetate through the mevalonic acid pathway. In the synthesis of terpenes, sequential additions of the 5-carbon isopentenyl pyrophosphate (IPP) produce terpenes with 10-, 15-, or 20-carbon atoms. Triterpenes are formed when two C15 (farnesyl) units join head to head to form the C30 molecule squalene. The subsequent biosynthesis of plant sterols is not yet fully understood, but the first step is a cyclization reaction to form cycloartenol. Using cycloartenol as a common precursor, there are probably multiple pathways leading to the several sterols found in plants. Decarboxylation and oxidation reactions are involved, as most common sterols have from 26 to 29 carbons and a single hydroxyl (-OH) group. It is thought that most sterols, with the exception of stigmasterol, may serve as precursors for various brassinosteroids. However, the pathway for the biosynthesis of brassinolide is best understood. The precursor to brassinolide is campesterol, a C28 sterol.

BRs act near their sites of synthesis and do not undergo long-distance transport

An important determinant of hormone responses in general is the extent and rate of hormone transport from the site of synthesis to the site of action. Exogenously applied 24-epibrassinolide (24-epiBL) undergoes long-distance transport from the root to the shoot. For example, when roots of cucumber, tomato, or wheat plants were treated with 14C-24-epiBL, the radioactivity was readily translocated to the shoot. Moreover, the dwarf phenotype of the BR-deficient *Arabidopsis* mutants could be restored (rescued) back to wild-type size, when grown on agar media supplemented with BL. In contrast, when 14C-24-epiBL was applied to the upper surface of a young cucumber leaf, it was readily taken up, but was only slowly transported out of the leaf. These results suggest that exogenous BRs are readily translocated from the root to the shoot, but are poorly translocated out of leaves.

BRs promote both cell proliferation and cell elongation

The growth-promoting effects of BRs are reflected in acceleration of both cell elongation and cell division. These were first characterized using the bean second-internode bioassay. The rice leaf lamina inclination bioassay is dependent on BR-induced cell expansion. Lamina inclination resembles the epinasty caused by ethylene. In response to BR, the cells on the adaxial (upper) surface of the leaf near the joint region expand more than the cells on the abaxial (lower) surface, causing the vertically oriented leaf to bend outward. An increase in cell wall loosening is required for BL-induced cell expansion on the adaxial side of the leaf. The stimulatory effect of BRs on growth is most pronounced in young, growing shoot tissues. The kinetics of cell expansion in response to nanomolar concentrations of BL differ from those of auxin-induced cell expansion. In soybean epicotyl sections, for example, BL begins to enhance the elongation rate after a 45-minute lag period, and reaches a maximum rate only after several hours of treatment. In contrast, auxin stimulates elongation after a 15-minute lag time and reaches a maximum rate within 45 minutes. These results suggest that the growth response to BRs may involve a slower pathway involving gene transcription, whereas the rapid response to auxin may not require gene transcription. In addition to cell elongation, BR also stimulates cell proliferation.

BRs promote root growth at low concentrations and inhibit root growth at high concentration

When applied exogenously, BRs promote root growth at low concentrations and inhibit root growth at high concentrations. The threshold concentration for inhibition depends on the activity of the BR analog used. The effects of BR on root growth are independent of both auxin and gibberellin action. An inhibitor of polar auxin transport, 2,3,5-triiodobenzoic acid (TIBA), does not prevent BR-induced growth. When BR and auxin are applied simultaneously, both the promotive and inhibitory effects on root growth are additive. Moreover, the reduced root growth phenotype of BR-deficient mutants is not reversed by gibberellin application. Taken together, these observations indicate that BR inhibition of root growth does not involve interactions with either auxin or GA. On the other hand, high concentrations of BR, like auxin, stimulate ethylene production, so it is possible that at least some of BR's inhibitory effects on root growth are due to ethylene. At low concentrations, BRs can also induce the formation of lateral roots. In these conditions, however, BRs and auxin act synergistically. The current model suggests that BRs promote lateral root development partially by influencing polar auxin transport.

BRs promote differentiation of the xylem and supress that of the phloem

BRs play an important role in vascular development, by promoting differentiation of the xylem and suppressing that of the phloem. This is evident in the impaired vasculature systems of BR mutants, which have a higher phloem-to-xylem ratio than the wild type. BR-deficient mutants also have a reduced number of vascular bundles

with irregular spacing between the bundles. In contrast, mutants overexpressing the BR receptor protein produce more xylem than the wild type.

BRs promote seed germination by interacting with ther hormones, such as GA and ABA

Seeds, like pollen grains, contain very high levels of BRs, and BRs promote seed germination as well. BRs promote seed germination by interacting with other plant hormones, although the molecular basis for these interactions is not known. It is well established that GA and abscisic acid (ABA) play positive and negative roles, respectively, in stimulating seed germination. BRs can enhance germination of tobacco seeds, independent of GA signaling. Moreover, BRs can rescue the delayed germination phenotype of both GA-deficient and GA-perception mutants, and BR mutants are more sensitive to the inhibition by ABA than the wild type. Thus, BRs can stimulate germination and are needed to overcome the inhibitory effect of ABA. As BRs are known to stimulate cell expansion and division, it is likely that BRs facilitate germination by stimulating the growth of the embryo.

4. Synthetic and microbial plant hormones in plant production

Hormones and other regulatory chemicals are now used in a variety of applications where it is desirable for commercial reasons to control some aspect of plant development.

Commercial application of auxins

Auxins have been used commercially in agriculture and horticulture for more than 50 years. The synthetic auxins are used in commercial applications largely because they are resistant to oxidation by enzymes that degrade IAA. In addition to their greater stability, the synthetic auxins are often more effective than IAA in specific applications. One of the most widespread uses of auxin encountered by the consumer is the use of 2,4-D in weed control. 2,4-D and other synthetic compounds, such as 2,4,5-T and dicamba, express auxin activity at low concentrations, but at higher concentrations are effective herbicides.

Indolebutyric acid and naphthaleneacetic acid are both widely used in vegetative propagation – the propagation of plants from stem and leaf cuttings. This application can be traced to the propensity for auxin to stimulate adventitious root formation. Generally marketed as “rooting hormone” preparations, the auxins, usually a synthetic auxin such as NAA or IBA, are mixed with an inert ingredient such as talcum powder. Stem cuttings are dipped in the powder prior to planting in a moist sand bed in order to encourage root formation.

4-CPA may be sprayed on tomatoes to increase flowering and fruit set while NAA is commonly used to induce flowering in pineapples. This latter effect is actually due to auxin-induced ethylene production. NAA is also used both to thin fruit set and prevent preharvest fruit drop in apples and pears. These seemingly opposite effects are dependent on timing the auxin application with the appropriate stage of flower and fruit development (**Figure 3.25**). Spraying in early fruit set, shortly after the flowers bloom, enhances abscission of the young fruits (again, due to auxin-induced ethylene production). Thinning is necessary in order to reduce the number of fruits and prevent too many small fruits from developing. Spraying as the fruit matures has the opposite effect, preventing premature fruit drop and keeping the fruit on the tree until it is fully mature and ready for harvest.

The use of synthetic auxins, especially the chlorinated forms, as herbicides has come under close scrutiny by environmental groups because of potential health hazards. 2,4,5-T, for example, has been banned in many jurisdictions because commercial preparations contain significant levels of dioxin, a highly carcinogenic chemical.

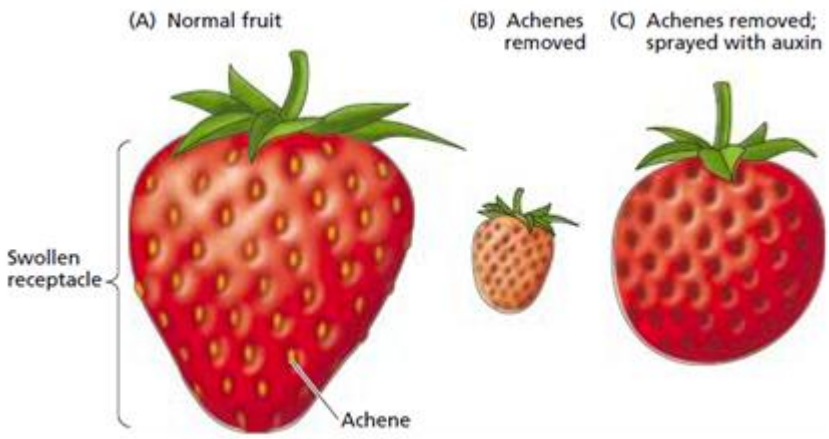


Figure 3.25 Auxin promotes fruit development that produced by achenes (*source: Taiz L., Zeiger E., 2010*)

Commercial use of gibberellins

The major uses of gibberellins (GA3), applied as a spray or dip, are to manage fruit crops, to malt barley, and to increase sugar yield in sugarcane. In some crops a reduction in height is desirable, and this can be accomplished by the use of gibberellin synthesis inhibitors.

Many of the table grapes grown in the United States are a genetically seedless variety that would naturally produce small fruit on very compact clusters. Almost all seedless grapes on the market are treated with GA3. It substitutes for the presence of seeds, which would normally be the source of native GAs for fruit growth. Repeated spraying with GA3 increases both rachis length (producing looser clusters) and fruit size (**Figure 3.26**). The increased rachis length prevents the cluster from being too compact, and this reduces the chance of fungal growth inside the cluster. Two to three additional applications of GA3 during fruit development are thought to increase berry size by enhancing the import of carbohydrates into the developing fruit.

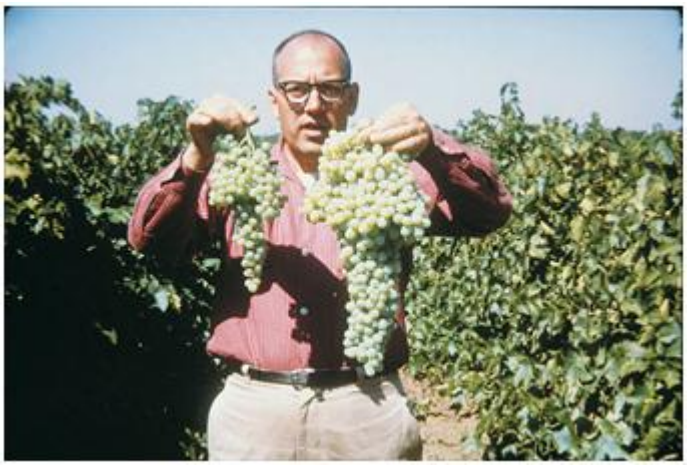


Figure 3.26 Gibberellin induces growth in Thompson's seedless grapes (left – control, right – sprayed with GA3) (*source: Taiz L., Zeiger E., 2010*)

Gibberellic acid is also used to boost cherry production. Sweet, bing cherries are sprayed 4 to 6 weeks before harvest to increase fruit size. Application of GA3 to tart cherries increases yield through enhanced bearing. Gibberellin A4 (GA4) is used to promote the fruit set of apple and pear trees. For example, in some apple cultivars the amount of fruit produced is often limited by biennial bearing, a phenomenon whereby the production of a heavy crop of fruit one year inhibits the subsequent production of flower buds, and hence, the yield of fruit the following year. The alternate bearing of some cultivars can be overcome by applying GA4 in the "off" year to promote the formation of flower buds, and subsequent fruit set. In regions of Europe where fruit set of apple and pear trees is often reduced by inclement weather at the time of pollination, the application of a hormone mixture can promote the production and subsequent growth of parthenocarpic (seedless) fruit. GA4/7 is also used on Golden Delicious apples to prevent abnormal cell divisions in the epidermal layer that

produce "russetting". Gibberellic acid is also applied to citrus crops, though the actual use depends on the particular crop. For example GA3 is sprayed onto oranges and tangerines to delay or prevent rind-aging, so that fruit can be harvested later without adverse effects on rind quality and appearance. For lemons and limes, GA3 synchronizes ripening and enhances fruit size.

Gibberellins from the embryo of germinating grains are necessary for the synthesis of α-amylase by the cells of the aleurone layer, which, in turn is necessary for the hydrolysis of starch within the endosperm. In the brewing industry, the production of beer relies on this hydrolytic breakdown of starch in barley grains to yield fermentable sugars, principally maltose, which are then subjected to fermentation by yeast. During fermentation, glycolytic enzymes from yeast break down the sugars, resulting in ethanol. In the multistep malting process, mature barley grains are steeped or soaked to allow them to imbibe water. Next, the grains are spread out to germinate, during which time the starch within the endosperm will be hydrolyzed by α-amylase allowing the embryo to begin to grow. This process of starch breakdown is referred to as "modification". Gibberellic acid may be applied during this time and will supplement the native GAs in the grain, enhance the production of α-amylase, and consequently, speed up the hydrolysis of starch.

Manipulation of cytokinins is a tool to alter agriculturally important strains

Some of the consequences of altering cytokinin function could be highly beneficial for agriculture if synthesis of the hormone can be controlled. Because leaf senescence is delayed in the cytokinin-overproducing plants, it should be possible to extend their photosynthetic productivity. Indeed, when an *ipt* gene is expressed in lettuce from a senescence-inducible promoter, leaf senescence is strongly retarded, similar to the results observed in tobacco.

In addition, cytokinin production could be linked to damage caused by predators. For example, tobacco plants transformed with an *ipt* gene under the control of the promoter from a wound-inducible protease inhibitor II gene were more resistant to insect damage. The tobacco hornworm consumed up to 70% fewer tobacco leaves in plants that expressed the *ipt* gene driven by the protease inhibitor promoter.

Manipulation of cytokinin also has the potential to increase grain yield in rice. Humans have unwittingly taken advantage of the promotive effect of cytokinin on the shoot apical meristem in their breeding of cultivated rice varieties. The rice varieties *japonica and indica* differ dramatically in their yield, with the latter generally producing more grains in their main panicle and ultimately a higher yield. The increased grain number in *indica* varieties has recently been linked to a decrease in the function of a cytokinin oxidase gene. As a consequence of the reduced function of this cytokinin oxidase in the *indica* varieties, cytokinin levels are higher in the inflorescence, which alters the inflorescence meristem such that it produces more reproductive organs, more seeds per plant, and ultimately a higher yield (**Figure 3.27**).

Figure 3.27 Cytokinin regulates grain yield in rice (*indica* variety has low number of cytokinin oxydase genes) (*source: Taiz L., Zeiger E., 2010*)

Large-scale cloning of plants by micropropagation

With a relatively small investment in space, technical support, and materials, tissue culture has made it possible to produce literally millions of high-quality, genetically uniform plants. The process is known as **micropropagation**. The most common technique is to place excised meristematic tissue on an artificial medium containing a cytokinin/auxin ratio that reduces apical dominance and encourages axillary bud development. The new shoots can be separated and sub-cultured to produce more axillary shoots, or placed on a medium that encourages rooting. Once roots appear, the plantlets can be planted out and allowed to develop into mature plants. Alternatively, excised tissues can be used to establish callus cultures, which may then be induced to form roots and shoots by manipulating the cytokinin/auxin ratio.

Micropropagation can also be an effective way to eliminate viruses and other pathogens and produce commercial quantities of pathogen-free propagules. The first plants to be mass-produced by tissue culture were virus-free orchids of the genus *Cymbidium*, but the technique has also been found useful for potato, lilies, tulips, and other species that are normally propagated vegetatively. Potato, for example, is vegetatively propagated through buds on the tubers, a system that readily transmits viruses to the next generation. Micropropagation of potato from meristem cultures has proven to be an effective way to isolate virus-free lines.

Micropropagation is also used extensively in the production of forest tree species. Here the propagules are generated primarily from cultures of axillary and adventitious buds; callusing and differentiation of new buds is rarely used. A similar approach has been applied successfully to cultivars of apple (*Malus*), peach (*Pyrus*), and pear (*Prunus*). Because most temperate fruits are highly heterozygous, they do not breed true from seed but are propagated by vegetative cuttings. Rooting of microcuttings in culture is now a routine procedure in many commercial laboratories.

The use of ethylene and brassinosteroids in plant production

As ethylene regulates many physiological processes in plant development, it is one of the most widely used plant hormones in agriculture. Auxins and ACC can trigger the natural biosynthesis of ethylene and in several cases are used in agricultural practice.

Ethephon (Ethrel) is the most widely used ethylene releasing compound

Because of its high diffusion rate, ethylene is very difficult to apply in the field as a gas, but this limitation can be overcome if an ethylene-releasing compound is used. The most widely used such compound is *Ethephon*, or 2-chloroethylphosphonic acid, which was discovered in the 1960s and is known by various trade names, such as *Ethrel*. Ethephon is sprayed in aqueous solution and is readily absorbed and transported within the plant. It releases ethylene slowly by a chemical reaction, allowing the hormone to exert its effects. It is used for:

- hastening fruit ripening of apple, tomato, and degreening of citrus;
- synchronized flowering and fruit set in pineapple, and accelerated abscission of flowers and fruits;
- inducing fruit thinning or fruit drop in cotton, cherry, and walnut;
- promoting female sex expression in cucumber, to prevent self-pollination and increase yield;
- inhibition of terminal growth of some plants in order to promote lateral growth and compact flowering stems.

Brassinosteroid (BR) application to crop plants is most effective under stress conditions

Brassinosteroids were discovered as a class of growth promoting hormones, and researchers immediately recognized their potential applications to agriculture. For the past 20 years, numerous small-scale studies have been conducted to test the ability of BRs to increase yields of crop plants. BL has been found to increase bean crop yield (based on the weight of seeds per plant) by about 45%, and to enhance the leaf weight of various lettuce varieties by 25%. Similar increases in the yields of rice, barley, wheat, and lentils have been observed. BL also promoted potato tuber growth and increased its resistance to infections. Tomato fruit set was also enhanced by BL. In addition to such small-scale studies, large-scale field trials using brassinosteroid derivatives have now been conducted in Japan, China, Korea, and Russia. The results of the field trials have been highly variable and appear to reflect the degree of stress under which the crop was grown. A crop grown under optimal conditions shows little effect of applied BR, while a crop grown under conditions of stress shows dramatic effects of BR application on yield.

Microbial plant hormones

Bacterial and fungal plant hormones

Some bacteria and fungi are intimately associated with higher plants. Many of these microorganisms produce and secrete substantial amounts of cytokinins and/or cause the plant cells to synthesize plant hormones, including cytokinins. The cytokinins produced by microorganisms include *trans*-zeatin, iP, *cis*-zeatin, and their ribosides, as well as 2-methylthio-derivatives of zeatin. Infection of plant tissues with these microorganisms can induce the tissues to divide and, in some cases, to form special structures, such as mycorrhizal arbuscules, in which the microorganism can reside in a mutualistic relationship with the plant.

In addition to the crown gall bacterium, *Agrobacterium tumefaciens*, other pathogenic bacteria may stimulate plant cells to divide. Without *Agrobacterium* infection, the wound-induced cell division would subside after a few days and some of the new cells would differentiate as a protective layer of cork cells or vascular tissue. However, *Agrobacterium* changes the character of the cells that divide in response to the wound, making them tumorlike. They do not stop dividing; rather, they continue to divide throughout the life of the plant to produce an unorganized mass of tumorlike tissue called a gall (**Figure 3.28**).

Figure 3.28 Tumor that formed on a tomato stem infected with the crown gall bacterium bearing cytokinin biosynthesis genes (*source: Taiz L., Zeiger E., 2010*)

Increased cytokinin, supplied by interacting bacteria, fungi, viruses, or insects, can cause an increase in the proliferation of the shoot apical meristem and/or the growth of lateral buds, which normally remain dormant. This proliferation, known as fasciation. often manifests as a phenomenon known as a witches' broom, so-called because these growths can resemble an old-fashioned straw broom. One well-studied causative agent of fasciation is *Rhodococcus fascians*. *R. fascians* produces several different cytokinins, including both cis-and trans-zeatin as well as their 2-methylthio-derivatives. This mixture of cytokinin species acts synergistically through the host's normal cytokinin signaling pathway to alter host development. *R. fascians* also secretes the auxin IAA, which contributes to the alteration in the growth of the host plant. Fasciation, which can also arise spontaneously by a mutation, is the basis for many of the horticultural dwarf conifers.

Microalgal plant hormones

There is accumulating evidence that both cyanobacteria and microalgae like to many seaweeds produce plant hormones, or demonstrate plant hormon-like activity. Recently, it is quite often that the beneficial effects of nitrogene-fixing cyanobacteria are explained with the influence of their PGRs instead of the increased available nitrogen for the rice plants.

The possibilities for applying microalgae in crop production has been investigated at the Faculty of Agricultural and Food Sciences, University of West Hungary, in Mosonmagyaróvár for several years. Indicator plants like potatoes and sugar beet proved the applicability of 3 algal strains out of many others, which we isolated (MACC-6, MACC-116, MACC-612). Small plot trials were carried out at ecological districts of the country, which show considerable differences, e.g. in counties Komárom, Szabolcs and Csongrád. We managed to influence the process of crop yielding capacity of potato and sugar beet with the investigated algal strains differently in method and size per habitat and year. We were able to influence the time of tillering and tuber

building, the number and size of tubers, which resulted in yield increase. At one of the trial sites in county Csongrád the strain MACC-612 showed a definite and well recognisable fungicide side effect in potatoes. We were able to influence the competition between beetroot and the foliage of sugar beet significantly and as a result of a longer active foliage life we could avoid harmful change of leaves even in climatic stress situations. With this successful treatment sugar beet yield per area unit increased and although the sugar content in percentage slightly decreased the absolute sugar yield increased as well (**Figure 3.29**). We applied microalgae in potato trials alone but they were applied as combination partners of fungicides in sugar beet. As a result the strains MACC-116 and MACC-612 can especially well be combined with strobilurin preparations.

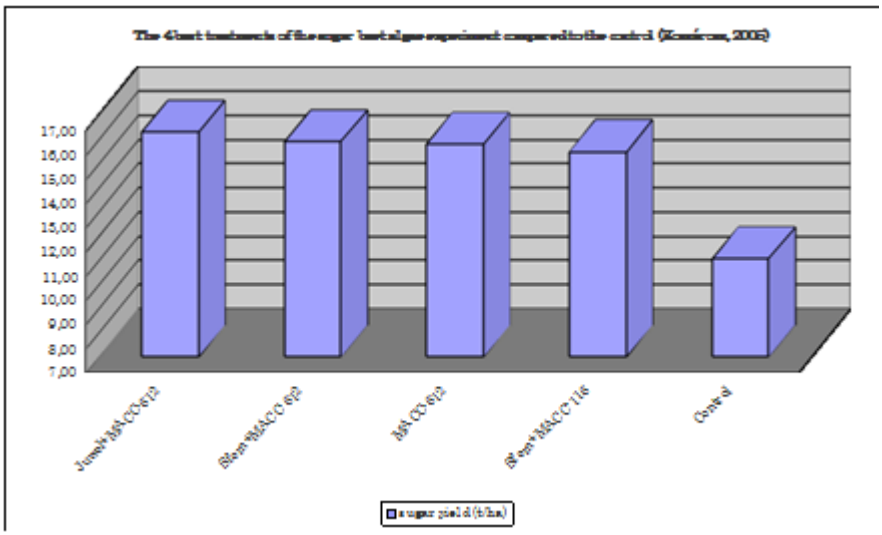


Figure 3.29 Sugar beet treatments with microalgae increase the sugar yield (*source: own result*)

Compounds of natural origins derived from higher plants are widely used in disease and pest control in ecological production. It is known, that seaweeds also contain chemical constituents, which has antimicrobial properties. In our experiments 255 microalgae strains were examined in vitro in agar gel diffusion test, to establish their effect on growth and development on plant pathogenic fungi. Four percent of tested algae strains showed fungicide, 59% fungistatic activity at least against one plant pathogen. The most effective strains were examined against a biotrophic plant pathogen, *Plasmopara viticola* a causal agent of grapevine downy mildew in vitro, using leaves and leaf discs. Inhibition effect of algae extract on the sporulation of pathogen reached the 100%. A field experiment was conducted in 2002, where MACC-14 strain was applied in 3, 5 and 10mg/ml concentration. The efficacy of algal suspension was about 50%.

In the tissue cultures of pea and tobacco the combination of extracellular compounds from microalgae and synthetic PGRs produced more fresh weight and regenerated shoot numbers than the control. The dilution of freeze dried biomass derived from MACC-304 and 612 has the same beneficial effect as the synthetic PGRs on tissue cultures of peas and tobacco.

According to the above mentioned own results we can state:

- bacteria, microalgae and cyanobacteria are able to produce several types of plant hormones;
- physiological status of cells (cell cycle) and environmental factors (light) influence the hormone production;
- highly reproducible results can be achieved by using synchronous cultures of microalgae, which can also explain the function of plant hormones in microalgae;
- broad leaf plants respond with yield increase on microalgal treatments.

Other synthetic growth regulators

Antiauxins inhibit the effects of auxins found in plants

Antiauxins are another class of synthetic auxin analogs. These compunds, such as α-(p-chlorophenoxy) isobutyric acid or PCIB, have little or no auxin activity but specially inhibit the effects of auxin. When applied

to plants, antiauxins may compete with IAA for specific receptors, thus inhibiting normal auxin action. One can overcome the inhibition of an antiauxin by adding excess IAA.

Several compounds have been synthesized that can act as auxin transport inhibitors, including NPA (l-N-naphthylphthalamic acid), TIBA (*2,3,5-triiodobenzoic acid*), CPD (2-carboxyphenyl-3-phenylpropane-l,3-dione), NOA (l-napthoxyacetic acid), 2-[4-(diethylamino) -Z-hydroxybenzoyl] benzoic acid, and gravacin. NPA, TIBA, CPD, and gravacin are auxin efflux inhibitors (AEIs), while NOA is an auxin influx inhibitor. Some AEIs, such as TIBA, have weak auxin activity and inhibit polar transport in part by competing with auxin at the efflux carrier site. Other AEIs, such as CPD, NPA, and gravacin interfere with auxin transport by binding to a regulatory site. Some inhibitors, such as gravacin, interfere more specifically with one type of transporter, while others, such as NPA, bind to and interfere with multiple proteins, some of which are only indirectly involved in auxin transport. Some natural compounds, primarily flavonoids, also function as auxin efflux inhibitors.

Synthetic antiauxins are used for:

- inhibition of shoot development of stored onions and potato tubers;
- inhibition of axillary shoot development in tobacco;
- control (inhibition) of lawn growth;
- promotion of sugarcane ripening;
- prevention against *Fusarium* diseases;
- promotion of stooling in cereals.

The inhibition of gibberellin biosynthesis also has commercial applications

The inhibition of gibberellin biosynthesis also has commercial applications. The growth of many stems can be reduced or inhibited by synthetic growth retardants or antigibberellins. These include AMO-1618, cycocel (or, CCC), Phosphon-D, ancymidol (known commercially as A-REST), and alar (or, B-nine). Growth retardants mimic the dwarfing genes by blocking specific steps in gibberellin biosynthesis, thus reducing endogenous gibberellin levels and suppressing internode elongation. These compounds have found significant commercial use, particularly in the production of ornamental plants. Growth retardants may be applied to potted plants either as a foliar spray or soil drench. Their principal effect is to reduce stem elongation, resulting in plants that are shorter and more compact, with darker green foliage. Flower size, however, is unaffected. Commercial flower growers have found these inhibitors useful in producing shorter, more compact poinsettias, lilies, and chrysanthemums, and other horticultural species. In some areas of the world, wheat tends to “lodge” near harvest time, that is, the plants become top-heavy with grain and fall over. Spraying the plants with antigibberellins produces a shorter, stiffer stem and thus prevents lodging. Antigiberellins also have been used to reduce the need for pruning of vegetation under power lines.

Inhibition of ethylene production and promotion preservation of fruits

Storage facilities developed to inhibit ethylene production and promote preservation of fruits have a controlled atmosphere of low O2 concentration and low temperature for the inhibition of ethylene biosynthesis. A relatively high concentration of CO2 (3 to 5%) prevents ethylene's action as a ripening promoter. Low pressure (vacuum) is used to remove ethylene and oxygen from the storage chambers, reducing the rate of ripening and preventing overripening. The ethylene binding inhibitor Ethylbloc® is increasingly being used to extend the shelf life of various climacteric fruits. Specific inhibitors of ethylene biosynthesis and action have proven useful in the postharvest preservation of flowers. Silver (Ag+) has been used extensively to increase the longevity of cut carnations and several other flowers. The potent inhibitor AVG retards fruit ripening and flower fading, but its commercial use has not yet been approved by regulatory agencies.

Decreased brassinosteroid (BR) synthesis or signaling lead to increased biomass and final seed yield

Reduced BR function can contribute to agriculture as well. For example, decreased BR synthesis or signaling in rice results in dwarfed plants with an erect leaf habit, which allows higher planting densities, leading to increased biomass and final seed yields. As researchers continue to explore BR's effects on plant development, additional applications of brassinosteroids to agriculture are bound to emerge.

5. Plant stress physiology

5.1. The basic concepts of plant stress, acclimation, and adaptation

Energy is an absolute requirement for the maintenance of structural organization over the lifetime of the organism. The maintenance of such complex order over time requires a constant through put of energy. The results in a constant flow of energy through all biological organisms, which provides the dynamic driving force for the performance of important maintenance processes such as cellular biosyntheses and transport to maintain its characteristic structure and organization as well as the capacity to replicate and grow. The maintenance of a steady-state results in a meta-stable condition called **homeostasis**.

Environmental modulation of homeostasis defined as biological stress

Any change in the surrounding environment may disrupt homeostasis. Environmental modulation of homeostasis may be defined as **biological stress**. Thus, it follows that **plant stress** implies some adverse effect on the physiology of a plant induced upon a sudden transition from some optimal environmental condition where homeostasis is maintained to some suboptimal condition which disrupts this initial homeostatic state. Thus, plant stress is a relative term since the experimental design to assess the impact of a stress always involves the measurement of a physiological phenomenon in a plant species under a suboptimal, stress condition compared to the measurement of the same physiological phenomenon in the same plant species under optimal conditions.

Plants respond to stress in several different ways

Plant stress can be divided into two primary categories. **Abiotic stress** is a physical (e.g., light, temperature) or chemical insult that the environment may impose on a plant. **Biotic stress** is a biological insult, (e.g., insects, disease) to which a plant may be exposed during its lifetime (**Figure 3.30**). Some plants may be injured by a stress, which means that they exhibit one or more metabolic dysfunctions. If the stress is moderate and short term, the injury may be temporary and the plant may recover when the stress is removed. If the stress is severe enough, it may prevent flowering, seed formation, and induce senescence that leads to plant death. Such plants are considered to be **susceptible**. Some plants escape the stress altogether, such as ephemeral, or short-lived, desert plants.

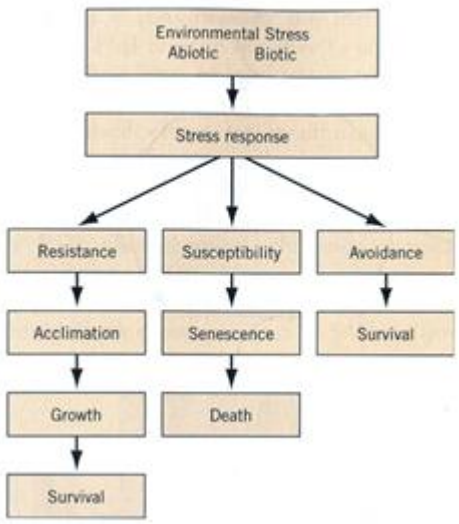


Figure 3.30 The effect of environmental stress on plant survival (*source: Hopkins W.G., Hüner N.P.A., 2009*)

Ephemeral plants germinate, grow, and flower very quickly following seasonal rains. They thus complete their life cycle during a period of adequate moisture and form dormant seeds before the onset of the dry season. In a similar manner, many arctic annuals rapidly complete their life cycle during the short arctic summer and survive over winter in the form of seeds. Because ephemeral plants never really experience the stress of drought or low temperature, these plants survive the environmental stress by **stress avoidance** (**Figure 3.30**). Avoidance

mechanisms reduce the impact of a stress, even though the stress is present in the environment. Many plants have the capacity to tolerate a particular stress and hence are considered to be **stress resistant** (**Figure 3.30**). Stress resistance requires that the organism exhibit the capacity to adjust or to acclimate to the stress.

Stress resistance requires that the organism exhibit the capacity to adjust or to acclimate to the stress

A plant stress usually reflects some sudden change in environmental condition. However, in stress-tolerant plant species, exposure to a particular stress leads to **acclimation** to that specific stress in a time-dependent manner (**Figure 3.31**). Thus, plant stress and plant acclimation are intimately linked with each other. The stress-induced modulation of homeostasis can be considered as the signal for the plant to initiate processes required for the establishment of a new homeostasis associated with the acclimated state. Plants exhibit stress resistance or stress tolerance because of their genetic capacity to adjust or to acclimate to the stress and establish a new homeostatic state over time. Furthermore, the acclimation process in stress-resistant species is usually reversible upon removal of the external stress (**Figure 3.31**).

The establishment of homeostasis associated with the new acclimated state is not the result of a single physiological process but rather the result of many physiological processes that the plant integrates over time, that is, integrates over the acclimation period. Plants usually integrate these physiological processes over a short-term as well as a long-term basis. The *short-term processes* involved in acclimation can be initiated within seconds or minutes upon exposure to a stress but may be transient in nature. That means that although these processes can be detected very soon after the onset of a stress, their activities also disappear rather rapidly. As a consequence, the lifetime of these processes is rather short. In contrast, *long-term processes* are less transient and thus usually exhibit a longer lifetime. However, the lifetimes of these processes overlap in time such that the short-term processes usually constitute the initial responses to a stress while the long-term processes are usually detected later in the acclimation process. Such a hierarchy of short- and long-term responses indicates that the attainment of the acclimated state can be considered a complex, time-nested response to a stress. Acclimation usually involves the differential expression of specific sets of genes associated with exposure to a particular stress. The remarkable capacity to *regulate gene expression* in response to environmental change in a time-nested manner is the basis of plant plasticity.

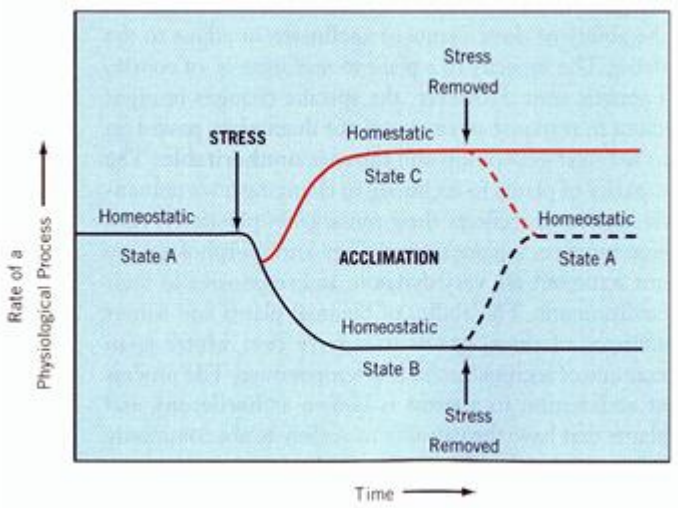


Figure 3.31 A schematic relationship between stress and acclimation (*source: Hopkins W.G., Hüner N.P.A., 2009*)

Adaptation and phenotypic plasticity

Plants have various mechanisms that allow them to survive and often prosper in the complex environments in which they live. **Adaptation** to the environment is characterized by genetic changes in the entire population that have been fixed by natural selection over many generations. In contrast, individual plants can also respond to changes in the environment, by directly altering their physiology or morphology to allow them to better survive the new environment. These responses require no new genetic modifications, and if the response of an individual improves with repeated exposure to the new environmental condition then the response is one of acclimation. Such responses are often referred to as **phenotypic plasticity**, and represent nonpermanent changes in the physiology or morphology of the individual that can be reversed if the prevailing environmental conditions change.

Individual plants may also show phenotypic plasticity that allows them to respond to environmental fluctuations

In addition to genetic changes in entire populations, individual plants may also show phenotypic plasticity; they may respond to fluctuations in the environment by directly altering their morphology and physiology. The changes associated with phenotypic plasticity require no new genetic modifications, and many are reversible. Both genetic adaptation and phenotypic plasticity can contribute to the plant's overall tolerance of extremes in their abiotic environment. As a consequence, a plant's physiology and morphology are not static but are very dynamic and responsive to their environment. The ability of biennial plants and winter cultivars of cereal grains to survive over winter is an example of acclimation to low temperature. The process of acclimation to a stress is known as **hardening** and plants that have the capacity to acclimate are commonly referred to as hardy species. In contrast, those plants that exhibit a minimal capacity to acclimate to a specific stress are referred to as nonhardy species.

Imbalances of abiotic factors have primary and secondary effects on plants

Plants may experience physiological stress when an abiotic factor is deficient or in excess (referred to as an imbalance). The deficiency or excess may be chronic or intermittent. Abiotic conditions to which native plants are adapted may cause physiological stress to non-native plants. Most agricultural crops, for example, are cultivated in regions to which they are not highly adapted. Field crops are estimated to produce only 22% of their genetic potential for yield because of suboptimal climatic and soil conditions.

Imbalances of abiotic factors in the environment cause *primary and secondary effects* in plants. Primary effects such as reduced water potential and cellular dehydration directly alter the physical and biochemical properties of cells, which then lead to secondary effects. These secondary effects, such as reduced metabolic activity, ion cytotoxicity, and the production of reactive oxygen species, initiate and accelerate the disruption of cellular integrity, and may lead ultimately to cell death. Different abiotic factors may cause similar primary physiological effects because they affect the same cellular processes. This is the case for water deficit, salinity, and freezing, all of which cause reduction in hydrostatic pressure (turgor pressure, Ψp) and cellular dehydration. Secondary physiological effects caused by different abiotic imbalances may overlap substantially. It is evident that imbalances in many abiotic factors reduce cell proliferation, photosynthesis, membrane integrity, and protein stability, and induce production of *reactive oxygen species (ROS)*, oxidative damage, and cell death.

5.2. The light-dependent inhibition of photosynthesis

As photoautotrophs, plants are dependent upon – and exquisitely adapted to – visible light for the maintenance of a positive carbon balance through photosynthesis. Higher energy wavelengths of electromagnetic radiation, especially in the ultraviolet range, can inhibit cellular processes by damaging membranes, proteins, and nucleic acids. However, even in the visible range, irradiances far above the light saturation point of photosynthesis cause high light stress, which can disrupt chloroplast structure and reduce photosynthetic rates, a process known as **photoinhibition**.

Photoinhibition by high light leads to the production of destructive forms of oxygen

Excess light excitation arriving at the PSII reaction center can lead to its inactivation by the direct damage of the D1 protein. Excess absorption of light energy by photosynthetic pigments also produces excess electrons outpacing the availability of NADP+ to act as an electron sink at PSI (**Figure 3.32**). The excess electrons produced by PSI lead to the production of reactive oxygen species (ROS), notably superoxide (O2□-). Superoxide and other ROS are low-molecular-weight molecules that function in signaling and, in excess, cause oxidative damage to proteins, lipids, RNA, and DNA. The oxidative stress generated by excessive ROS destroys cellular and metabolic functions and leads to cell death.

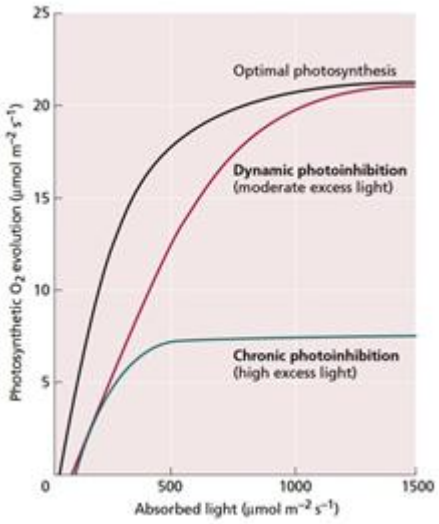


Figure 3.32 Changes in the light-response curves of photosynthesis caused by photoinhibition (*source: Taiz L., Zeiger E., 2002*)

5.3. Temperature stress

Mesophytic plants (terrestrial plants adapted to temperate environments that are neither excessively wet nor dry) have a relatively narrow temperature range of about 10°C for optimal growth and development. Outside of this range, varying amounts of damage occur, depending on the magnitude and duration of the temperature fluctuation. In this section we will discuss three types of temperature stress: high temperatures, low temperatures above freezing, and temperatures below freezing. Most actively growing tissues of higher plants are tillable to survive extended exposure to temperatures above 45°C or even short exposure to temperatures of 55°C or above. However, nongrowing cells or dehydrated tissues (e.g., seeds and pollen) remain viable at much higher temperatures. Pollen grains of some species can survive 70°C and some dry seeds can tolerate temperatures as high as 120°C.

Most plants with access to abundant water are able to maintain leaf temperatures below 45°C by evaporative cooling, even at elevated ambient temperatures. However, high leaf temperatures combined with minimal evaporative cooling causes heat stress. Leaf temperatures can rise to 4 to 5°C above ambient air temperature in bright sunlight near midday, when soil water deficit causes partial stomatal closure or when high relative humidity reduces the gradient driving evaporative cooling. Increases in leaf temperature during the day can be more pronounced in plants experiencing drought and high irradiance from direct sunlight.

Temperature stress can result in damaged membranes and enzymes

Plant membranes consist of a lipid bilayer interspersed with proteins and sterols, and any abiotic factor that alters membrane properties can disrupt cellular processes. The physical properties of the lipids greatly influence the activities of the integral membrane proteins, including H+-pumping ATPases, carriers, and channel-forming proteins that regulate the transport of ions and other solutes. High temperatures cause an increase in the fluidity of membrane lipids and a decrease in the strength of hydrogen bonds and electrostatic interactions between polar groups of proteins within the aqueous phase of the membrane. High temperatures thus modify membrane composition and structure, and can cause leakage of ions. High tempeatures can also lead to a loss of the three-dimensional structure required for correct function of enzymes or structural cellular components, thereby leading to loss of proper enzyme structure and activity. Misfolded proteins often aggregate and precipitate, creating serious problems within the cell.

Temperature stress can inhibit photosynthesis

Photosynthesis and respiration are both inhibited by temperature stress. Typically, photosynthetic rates are inhibited by high temperatures to a greater extent than respiratory rates. Although chloroplast enzymes such as rubisco, rubisco activase, NADP-G3P dehydrogenase, and PEP carboxylase become unstable at high temperatures, the temperatures at which these enzymes began to denature and lose activity are distinctly higher than the temperatures at which photosynthetic rates begin to decline. This would indicate that the early stages of

heat injury to photosynthesis are more directly related to changes in membrane properties and to uncoupling of the energy transfer mechanisms in chloroplasts.

This imbalance between photosynthesis and respiration is one of the main reasons for the deleterious effects of high temperatures. On an individual plant, leaves growing in the shade have a lower temperature compensation point than leaves that are exposed to the sun (and heat). Reduced photosynthate production may also result from stress-induced stomatal closure, reduction in leaf canopy area, and regulation of assimilate partitioning.

Freezing temperatures cause ice crystal formation and dehydration

Freezing temperatures result in intra- and extracellular ice crystal formation. Intracellular ice formation physically shears membranes and organelles. Extracellular ice crystals, which usually form before the cell contents freeze, may not cause immediate physical damage to cells, but they do cause cellular dehydration. This is because ice formation substantially lowers the water potential (Ψw) in the apoplast, resulting in a gradient from high Ψw in the symplast to low Ψw in the apoplast. Consequently, water moves from the symplast to the apoplast, resulting in cellular dehydration. Cells that are already dehydrated, such as those in seeds and pollen, are relatively less affected by ice crystal formation. Ice usually forms first within the intercellular spaces and in the xylem vessels, along which the ice can quickly propagate. This ice formation is not lethal to hardy plants, and the tissue recovers fully if warmed. However, when plants are exposed to freezing temperatures for an extended period, the growth of extracellular ice crystals leads to physical destruction of membranes and excessive dehydration.

5.4. Imbalances in soil minerals

Imbalances in the mineral content of soils can affect plant fitness either indirectly, by affecting plant nutritional status or water uptake, or directly, through toxic effects on plant cells.

Soil mineral content can result in plant stress in various ways

Several anomalies associated with the elemental composition of soils can result in plant stress, including high concentrations of salts (e.g., Na^+ and Cl^-) and toxic ions (e.g., As and Cd), and low concentrations of essential mineral nutrients, such as Ca^{2+}, Mg^{2+}, N, and P. The term salinity is used to describe excessive accumulation of salt in the soil solution. **Salinity stress** has two components: nonspecific osmotic stress that causes water deficits, and specific ion effects resulting from the accumulation of toxic ions, which disturb nutrient acquisition and result in cytotoxicity. Salt-tolerant plants genetically adapted to salinity are termed *halophytes*, while less salt-tolerant plants that are not adapted to salinity are termed *glycophytes*.

Soil salinity occurs naturally and as the result of improper water management practices

In natural environments, there are many causes of salinity. Terrestrial plants encounter high salinity close to the seashore and in estuaries where seawater and freshwater mix or replace each other with the tides. The movement of seawater upstream into rivers can be substantial, depending on the strength of the tidal surge. Far inland, natural seepage from geologic marine deposits can wash salt into adjoining areas. Evaporation and transpiration remove pure water (as vapor) from the soil, concentrating the salts in the soil solution. Soil salinity is also increased when water droplets from the ocean disperse over land and evaporate.

Human activities also contribute to soil salinization. Improper water management practices associated with intensive agriculture can cause substantial salinization of croplands. In many areas of the world, salinity threatens the production of staple foods. Irrigation water in semiarid and arid regions is often saline. Only halophytes, the most salt-tolerant plants, can tolerate high levels of salts. Glycophytic crops cannot be grown with saline irrigation water.

Saline soils are often associated with high concentrations of NaCl, but in some areas Ca^{2+}, Mg^{2+}, and SO_4^- are also present in high concentrations in saline soils. High Na^+ concentrations that occur in sodic soils (soils in which Na^+ occupies $\geqslant$10% of the cation exchange capacity) not only injure plants but also degrade the soil structure, decreasing porosity and water permeability. Salt incursion into the soil solution causes water deficits in leaves and inhibits plant growth and metabolism.

High cytosolic Na^+ and Cl^- denature proteins and destabilize membranes

The most widespread example of a specific ion effect is the cytotoxic accumulation of Na+ and Cl- ions under saline conditions. Under non-saline conditions, the cytosol of higher plant cells contains about 100 mM K+ and less than 10 mM Na+, an ionic environment in which enzymes are optimally functional. In saline environments, cytosolic Na+ and Cl- increase to more than 100 mM, and these ions become cytotoxic. High concentrations of salt cause protein denaturation and membrane destabilization by reducing the hydration of these macromolecules. However, Na+ is a more potent denaturant than K+.

At high concentrations, apoplastic Na+ also competes for sites on transport proteins that are necessary for high-affinity uptake of K+, an essential macronutrient. Further, Na+ displaces Ca2+ from sites on the cell wall, reducing Ca2+ activity in the apoplast and resulting in greater Na+ influx, presumably through nonselective cation channels. Reduced apoplastic Ca2+ concentrations caused by excess Na+ may also restrict the availability of Ca2+ in the cytosol. Since cytosolic Ca2+ is necessary to activate Na+ detoxification via efflux across the plasma membrane, elevated external Na+ has the ability to block its own detoxification.

5.5. Developmental and physiological mechanisms against environmental stress

Plants can modify their life cycles to avoid abiotic stress

One way plants can adapt to extreme environmental conditions is through modification of their life cycles. For example, annual desert plants have short life cycles: they complete them during the periods when water is available, and are dormant (as seeds) during dry periods. Deciduous trees of the temperate zone shed their leaves before the winter so that sensitive leaf tissue is not damaged by cold temperatures. During less predictable stressful events (e.g., a summer of significant but erratic rainfall) the growth habits of some species may confer a degree of tolerance to these conditions. For example, plants that can grow and flower over an extended period (*indeterminate growth*) are often more tolerant to erratic environmental extremes than plants that develop preset numbers of leaves and flower over only very short periods (*determinate growth*).

Phenotypic changes in leaf structure and behavior are important stress responses

Because of their roles in photosynthesis, leaves (or their equivalent) are crucial to the survival of a plant. To function, leaves must be exposed to sunlight and air, but this also makes them particularly vulnerable to environmental extremes. Plants have thus evolved various mechanisms that enable them to avoid or mitigate the effects of abiotic extremes to leaves. Such mechanisms include changes in leaf area, leaf orientation, trichomes, and the cuticle.

Turgor reduction is the earliest significant biophysical effect of water deficit. As a result, turgor-dependent processes such as *leaf expansion* and root elongation are the most sensitive to water deficits. When water deficit develops slowly enough to allow changes in developmental processes, it has several effects on growth, one of which is a limitation of leaf expansion. Because leaf expansion depends mostly on cell expansion, the principles that underlie the two processes are similar. Inhibition of cell expansion results in a slowing of leaf expansion early in the development of water deficits. The resulting smaller leaf area transpires less water, effectively conserving a limited water supply in the soil over a longer period. Altering *leaf shape* is another way that plants can reduce leaf area. Under conditions of water, heat, or salinity extremes, leaves may be narrower or may develop deeper lobes during development (**Figure 3.33**). The result is a reduced leaf surface area and therefore, reduced water loss and heat load (defined as amount of heat loss [cooling] required to maintain a leaf temperature close to air temperature). For protection against overheating during water deficit, the leaves of some plants may orient themselves away from the sun. *Leaf orientation* may also change in response to low oxygen availability.

Figure 3.33 Altered leaf shape can occur in response to environmental changes: leaf from outside (left) and inside (right) of a tree canopy (*source: Taiz L., Zeiger E., 2010*)

Plants can regulate stomatal aperture in response to dehydration stress

The ability to control stomatal aperture allows plants to respond quickly to a changing environment, for example to avoid excessive water loss or limit uptake of liquid or gaseous pollutants through stomata. Stomatal opening and closing is modulated by uptake and loss of water in guard cells, which changes their turgor pressure. Although guard cells can lose turgor as a result of a direct loss of water by evaporation to the atmosphere, stomatal closure in response to dehydration is almost always an active, energy-dependent process rather than a passive one. Abscisic acid (ABA) mediates the solute loss from guard cells that is triggered by a decrease in the water content of the leaf. Plants constantly modulate the concentration and cellular localization of ABA, and this allows them to respond quickly to environmental changes, such as fluctuations in water availability.

Plants adjust osmotically to drying soil by accumulating solutes

Osmotic adjustment is the capacity of plant cells to accumulate solutes and use them to lower Ψw during periods of osmotic stress. The adjustment involves a net increase in solute content per cell that is independent of the volume changes that result from loss of water. The decrease in ΨS (= osmotic potential) is typically limited to about 0.2 to 0.8 MPa, except in plants adapted to extremely dry conditions.

There are two main ways by which **osmotic adjustment** can take place. A plant may *take up ions* from the soil, or *transport ions* from other plant organs to the root, so that the solute concentration of the root cells increases. For example, increased uptake and accumulation of K+ will lead to decreases in ΨS due to the effect of the potassium ions on the osmotic pressure within the cell. This is a common event in saline areas, where ions such as potassium and calcium are readily available to the plant. The accumulation of ions during osmotic adjustment is predominantly restricted to the vacuoles, where the ions are kept out of contact with cytosolic enzymes or organelles.

When ions are compartmentalized in the vacuole, other solutes must accumulate in the cytoplasm to maintain water potential equilibrium within the cell. These solutes are called *compatible solutes* (or *compatible osmolytes*). Compatible solutes are organic compounds that are osmotically active in the cell, but do not destabilize the membrane or interfere with enzyme function, as high concentrations of ions can. Plant cells can hold large concentrations of these compounds without detrimental effects on metabolism. Common compatible solutes include amino acids such as proline, sugar alcohols such as mannitol, and quaternary ammonium compounds such as glycine betaine.

Phytochelatins chelate certain ions, reducing their reactivity and toxicity

Chelation is the binding of an ion with at least two ligating atoms within a chelating molecule. Chelating molecules can have different atoms available for ligation, such as sulfur (S), nitrogen (N), or oxygen (O), and these different atoms have different affinities for the ions they chelate. By wrapping itself around the ion it binds to form a complex, the chelating molecule renders the ion less chemically active, thereby reducing its potential toxicity. The complex is then usually translocated to other parts of the plant, or stored away from the cytoplasm (typically in the vacuole). **Phytochelatins** are low-molecular-weight thiols consisting of the amino acids

glutamate, cysteine, and glycine, with the general form of (γ-Glu-Cys)nGly. The thiol groups act as ligands for ions of trace elements such as Cd and As. Once formed, the phytochelatin-metal complex is transported into the vacuole for storage.

Many plants have the capacity to acclimate to cold temperature

The ability to tolerate freezing temperatures under natural conditions varies greatly among tissues. Seeds and other partially dehydrated tissues, as well as fungal spores, can be kept indefinitely at temperatures near absolute zero (0 K, or -273°C), indicating that these very low temperatures are not intrinsically harmful. Hydrated, vegetative cells can also retain viability at freezing temperatures, provided that ice crystal formation can be restricted to the intercellular spaces and cellular dehydration is not too extreme.

Temperate plants have the capacity for *cold acclimation* – a process whereby exposure to low but nonlethal temperatures (typically above freezing) increases the capacity for low temperature survival. Cold acclimation in nature is induced in the early autumn by exposure to short days and nonfreezing, chilling temperatures, which combine to stop growth. A diffusible factor that promotes acclimation, most likely ABA, moves from leaves via the phloem to overwintering stems. ABA accumulates during cold acclimation and is necessary for this process.

Plants survive freezing temperatures by limiting ice formation

During rapid freezing, the protoplast, including the vacuole, may supercool; that is, the cellular water remains liquid because of its solute content, even at temperatures several degrees below its theoretical freezing point. Supercooling is common to many species of the hardwood forests. Cells can supercool to only about -40°C, the temperature at which ice forms spontaneously. Spontaneous ice formation sets the low-temperature limit at which many alpine and subarctic species that undergo deep supercooling can survive. It may also explain why the altitude of the timberline in mountain ranges is at or near the -40°C minimum isotherm. Several specialized plant proteins, termed **antifreeze proteins**, limit the growth of ice crystals through a mechanism independent of lowering of the freezing point of water. Synthesis of these antifreeze proteins is induced by cold temperatures. The proteins bind to the surfaces of ice crystals to prevent or slow further crystal growth.

Cold-resistant plants tend to have membranes with more unsaturated fatty acids

As temperatures drop, membranes may go through a phase transition from a flexible liquid-crystalline structure to a solid gel structure. The phase transition temperature varies with species (tropical species: 10-12°C; apples: 3-10°C) and the actual lipid composition of the membranes. Chilling-resistant plants tend to have membranes with more unsaturated fatty acids. Chilling-sensitive plants, on the other hand, have a high percentage of saturated fatty acid chains, and membranes with this composition tend to solidify into a semicrystalline state at a temperature well above 0°C. Prolonged exposure to extreme temperatures may result in an altered composition of membrane lipids, a form of acclimation. Certain transmembrane enzymes can alter lipid saturation, by introducing one or more double bonds into fatty acids. This modification lowers the temperature at which the membrane lipids begin a gradual phase change from fluid to semicrystalline form and allows membranes to remain fluid at lower temperatures, thus protecting the plant against damage from chilling.

A large variety of heat shock proteins can be induced by different environmental conditions

Under environmental extremes, protein structure is sensitive to disruption. Plants have several mechanisms to limit or avoid such problems, including osmotic adjustment for maintenance of hydration and chaperone proteins that physically interact with other proteins to facilitate protein folding, reduce misfolding and aggregation, and stabilize protein tertiary structure. In response to sudden 5 to 10°C increases in temperature, plants produce a unique set of chaperone proteins referred to as **heat shock proteins** (HSPs). Cells that have been induced to synthesize HSPs show improved thermal tolerance and can tolerate subsequent exposure to temperatures that otherwise would be lethal. Heat shock proteins are also induced by widely different environmental conditions, including water deficit, ABA treatment, wounding, low temperature, and salinity. Thus, cells that have previously experienced one condition may gain cross-protection against another.

During mild or short-term water shortage, photosynthesis is strongly inhibited, but phloem translocation is unaffected until the shortage becomes severe

Changes in the environment may stimulate shifts in metabolic pathways. When the supply of O2 is insufficient for aerobic respiration, roots first begin to ferment pyruvate to lactate through the action of lactate dehydrogenase; this recycles NADH to NAD+, allowing the maintenance of ATP production through glycolysis. Production of lactate (lactic acid) lowers the intracellular pH, inhibiting lactate dehydrogenase and

activating pyruvate decarboxylase. These changes in enzyme activity quickly lead to a switch from lactate to ethanol production. The net yield of ATP in fermentation is only 2 moles of ATP per mole of hexose sugar catabolized (compared with 36 moles of ATP per mole of hexose respired in aerobic respiration). Thus, injury to root metabolism by O2 deficiency originates in part from a lack of ATP to drive essential metabolic processes such as root absorption of essential nutrients.

Water shortage decreases both photosynthesis and the consumption of assimilates in the expanding leaves. As a consequence, water shortage indirectly decreases the amount of photosynthate exported from leaves. Because phloem transport depends on pressure gradients, decreased water potential in the phloem during water deficit may inhibit the movement of assimilates. The ability to continue translocating assimilates is a key factor in almost all aspects of plant resistance to drought.

Chapter 5. References

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Chapter 6. Questions

What is the importance of water in a plant's life?

What kind of driving forces are involved in water movement?

What are the components of plant water potential?

How plant water potential can be measured?

What is the role of root hairs in water uptake?

How the water is absorbed and moved from soil to plant's canopy?

What transpiration types exist in plant kingdom?

What are the main characteristics of plant water status?

A decrease or cessation of leaf expansion is an early response to water stress. Provide a mechanism for this response.

Explain the role of the stomatal response to abscisic acid in plant tolerance to water stress.

What does a plant need to grow from seed and complete its life cycle?

What is an essential element? How many have been identified?

What is a mineral deficiency? How can a mineral deficiency be recognized?

How can farmers benefit from nutrient analysis?

What is the importance of micorrhizal fungi?

What is meant by the term “passive transport” and “active transport”?

Both membrane channels and carriers show changes in protein conformation. What is the role of such conformation changes (a) in channels, and (b) in carriers?

In the transport of an ion from the soil solution to the xylem, what is the minimum number of times it must cross a cell membrane?

Describe the pressure-flow model of translocation in the phloem.

What is the relation between the electromagnetic spectrum of solar radiation and the absorption spectrum of chlorophyll?

Describe the two photosystems and provide two lines of experimental evidence that led to their discovery.

What is the role of electron transport in oxygen-evolving photosynthesis? Describe the path traveled by an electron in the electron transport process.

Can ATP synthesis take place in thylakoid membranes kept in the dark? Explain your answer.

Describe the different types of carbon reactions of photosynthesis in higher plants.

If the level of atmospheric CO2 were to double, how would the photosynthesis be affected? Explain your answer.

How many environmental factors can limit photosynthesis at one time?

Discuss the main functions of secondary metabolites in plants and relate these functions to the sites of accumulation of secondary compounds in the plant.

What are terpenes chemically, and how are they synthesized?

How do alkaloids differ structurally from the other secondary compounds? Given their biological effects, how might they function ecologically?

Do plants, like animals, have an immune response to pathogens?

Distinguish between growth, differentiation, and development.

Describe the significance of meristems.

What is the process of seed formation from a fertilized egg cell?

What is the importance of programmed cell death (PCD) in a dicot plant's life?

What characteristics contribute strength and rigidity to a cell wall?

What limitations does the cell wall place on the growth of plant cell?

What is the physiological significance of physiological ecotypes, or photoperiodic races within a species that are chracterized by different critical daylengths?

Why is it necessary for a hormone to be rapidly turned over?

Can you suggest the physiological advantage of the accumulation of auxin conjugates in some seeds?

How is the polar auxin transport accomplished?

What are the major physiological role of auxin?

Describe the main physiological effects of gibberellins and cytokinins in a plant's life.

What is the evidence that cytokinins are required for the maintenance of the shoot apical meristems?

What is the apparent role of gibberellins in the shoot apical meristems?

What are the agronomical purposes of gibberellins and cytokinins?

What unique problems are related to the study of ethylene as a plant hormone? What is the evidence that ABA mediates responses to water stress?

How are gibberellins and brassinosteroids related biosynthetically?

Describe the hormonal changes that occur during seed development, maturation, and germination.

Discuss the evidence for the role of auxin in the following physiological phenomena: apical dominance, lateral and adventitious roots, leaf abscission, floral bud development, fruit development.

Give several examples of the effects of gibberellins on plant development. Have any of these responses been used commercially?

Discuss five physiological responses regulated by ethylene.

What are the sources of natural plant hormones used in plant production?

Define plant stress, stress tolerance, and acclimation to stress.

If plants require light for photosynthesis, explain why plants can be exposed to too much light.

What is osmotic stress? Explain how plant cells use compatible solutes to achieve osmotic adjustment.

Why do cold-acclimated winter cereals exhibit an increased tolerance to photoinhibition?

What are heat shock proteins?