PLANT PHYSIOLOGY

M.Sc. BOTANY

FIRST YEAR, SEMESTER-II, PAPER-II

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M.Sc. BOTANY: Plant Physiology

First Edition : 2025

No. of Copies :

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Published by:

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Acharya Nagarjuna University

Printed at:

FOREWORD

Since its establishment in 1976, Acharya Nagarjuna University has been forging ahead in the path of progress and dynamism, offering a variety of courses and research contributions. I am extremely happy that by gaining 'A+' grade from the NAAC in the year 2024, Acharya Nagarjuna University is offering educational opportunities at the UG, PG levels apart from research degrees to students from over 221 affiliated colleges spread over the two districts of Guntur and Prakasam.

The University has also started the Centre for Distance Education in 2003-04 with the aim of taking higher education to the door step of all the sectors of the society. The centre will be a great help to those who cannot join in colleges, those who cannot afford the exorbitant fees as regular students, and even to housewives desirous of pursuing higher studies. Acharya Nagarjuna University has started offering B.Sc., B.A., B.B.A., and B.Com courses at the Degree level and M.A., M.Com., M.Sc., M.B.A., and L.L.M., courses at the PG level from the academic year 2003-2004 onwards.

To facilitate easier understanding by students studying through the distance mode, these self-instruction materials have been prepared by eminent and experienced teachers. The lessons have been drafted with great care and expertise in the stipulated time by these teachers. Constructive ideas and scholarly suggestions are welcome from students and teachers involved respectively. Such ideas will be incorporated for the greater efficacy of this distance mode of education. For clarification of doubts and feedback, weekly classes and contact classes will be arranged at the UG and PG levels respectively.

It is my aim that students getting higher education through the Centre for Distance Education should improve their qualification, have better employment opportunities and in turn be part of country's progress. It is my fond desire that in the years to come, the Centre for Distance Education will go from strength to strength in the form of new courses and by catering to larger number of people. My congratulations to all the Directors, Academic Coordinators, Editors and Lesson-writers of the Centre who have helped in these endeavors.

Prof. K. Gangadhara Rao
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M.Sc. BOTANY

FIRST YEAR, Semester-II, Paper-II

202BO24: PLANT PHYSIOLOGY

SYLLABUS

UNIT-I

Membrane transport and translocation of water and solutes: The structure and properties of water; water transport processes (diffusion, bulk flow, osmosis, water potential, components of water potential); Mechanism of water transport through xylem; Solute transport by active and passive mechanisms. Structure and properties of membrane transport proteins.

UNIT - II

Water loss by transpiration; Mechanism of stomatal movements; antitranspirants. Sensory Photobiology: Historical discovery of phytochromes, structure and function of phytochrome, photochemical and biochemical properties of phytochrome, phytochrome induced plant responses, molecular mechanism of action of phytochrome in gene expression, Cryptochrome and its role in photomorphogenesis.

UNIT - III

The flowering process- Photoperiodism and its significance, initiation of flower primordia, flowering stimulus, vernalization, endogenous clock and its regulation. plant growth regulators: Physiological effects and mode of action of auxins, gibberellins, cytokinins, ethylene, abscisic acid, brassinosteroids, jasmonic acid and salicylic acid.

UNIT-IV

Signal transduction: Over view, receptors and G proteins, second messengers, two component sensor regulator system in bacteria and plants, signal transduction and gene expression. Essential nutrients, deficiencies and plant disorders.

UNIT-V Stress Physiology: Water stress, salt stress, temperature stress (HSP), biotic stress (HR and SAR)' heavy metal stress; Stress avoidance and tolerance mechanisms; Structural, physiological, biochemical and molecular responses of plants to environmental stress; Reclamation of saline and heavy metal contaminated soils.

REFERENCE BOOKS:

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Paper Code: 202BO24

CENTRE FOR DISTANCE EDUCATION
ACHARYA NAGARJUNA UNIVERSITY
M.Sc. DEGREE EXAMINATIONS (2025)
Second Semester
BOTANY
PAPER 2.2: **Plant Physiology**

Time 3 Hours Max Marks: 70

Answer All Questions
Each Question carries equal marks (5X14=70)

UNIT –I

1. (a) Give a detailed note on different types of membrane transport mechanisms.

Or

(b) Give a detailed note on the structure and properties of membrane transport proteins.

UNIT –II

2. (a) Give a detailed note on the mechanism of stomatal movements.

Or

(b) Give a detailed note on the structure, functions and properties of phytochromes

UNIT –III

3. (a) Give a detailed note on the flowering process

Or

(b) Give a detailed note on vernalization

UNIT –IV

4. (a) Give a detailed note on signal transduction and gene expression

Or

(b) Give a detailed note on the two-component sensor regulator system

UNIT –V

5. (a) Give a detailed note on different types of stress.

Or

(b) Give a detailed note on different types of responses to environmental stress

CONTENTS

LESSON- 1

PLANT WATER RELATIONS

OBJECTIVE:

In this lesson, the unique physicochemical properties of water and its transport by diffusion and bulk flow in biological systems, water potential concept and its components are discussed.

1.1 INTRODUCTION

Water is the most dominant constituent of living organisms, and it constitute 70 percent of the weight in woody plants and 20 % in certain desiccation tolerant plants and only 5% in dry seeds. The desiccated plants can be revived in their metabolic activity only after the water content reaches normal levels.

Since water has unique physical and chemical properties to drive many important processes in the physiology of plants. This thermal property of water is important because most of the biochemical reactions occur in an aqueous medium only. The thermal properties of water also contribute to temperature regulation. Water also has excellent solvent properties which enable the water to act as a suitable medium for the uptake and distribution of mineral

nutrients and other solutes required for growth. In addition, water is a transparent medium. This property of water permits visible light to penetrate the aqueous medium of cells to power photosynthesis or control development.

Plants actively carrying out photosynthesis are generally subjected to substantial water loss through evaporation from the leaf surfaces. Plants, however, take up large quantities of water from the soil to allow all cells to be present in turgid condition.

1.2 PHYSICAL AND CHEMICAL PROPERTIES OF WATER - HYDROGEN BONDING

Water consists of an oxygen atom covalently bonded to two hydrogen atoms. The oxygen is strongly electronegative which means that it tends to attract electrons. So that in the water molecule, oxygen tends to draw electrons away from the hydrogen. As a result, the two electrons that make up the O-H bond in the water molecule, are closer to the oxygen nucleus than to hydrogen resulting in oxygen atom carrying a partial negative charge and a corresponding positive charge is shared between the two hydrogen atoms. This asymmetric electron distribution makes water a polar molecule. This separation of negative and positive charges generates a strong mutual (electrical) attraction between adjacent water molecules or between water and other polar molecules. This is called hydrogen bonding (Figure 1.1) and this is largely responsible for many unique properties of water compared with other molecules of similar molecular size. Hydrogen bonding, for example, is the basis for hydration shells that form biologically important macromolecules such as proteins. nucleic acids and carbohydrates. The hydrogen bond results from the electrostatic attraction between the partial positive charge on one molecule and the positive negative charge on the next.

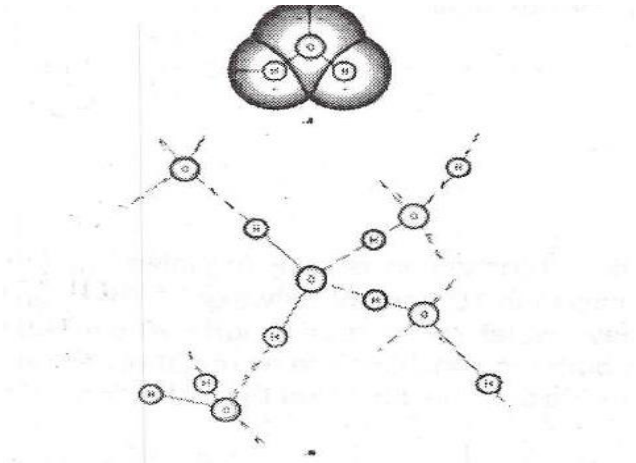

Figure 1.1 (A) Schematic structure of water molecules.

Thermal Properties of Water

The thermal properties of water that result from hydrogen bonding are among the most biologically important. Water remains in a liquid state over the range of temperatures most compatible with life. Boiling and melting points are generally related to molecular size. Water being a small molecule might be expected to exist primarily in the vapor state at temperatures encountered over most of the earth. However, the melting and boiling points of water are higher than expected when compared with other molecules of similar sizes such as ammonia and methane. This is because the presence of oxygen in the water induces polarity

and opportunity to form hydrogen bonds. Specific heat is defined as the amount of energy required to raise the temperature of one gram of substance by 1°C. The thermal capacity or specific heat of water is 4.184J s:' °C-1. This value is higher than that of any substance except liquid ammonia. In addition, liquid water also has 'a heigh thermal conductivity' (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. This property of water, therefore, enables them to maintain constant internal temperature under conditions of localized overheating due to the heat of biochemical reactions and temperature variations in the surrounding environment.

Energy is required to cause changes in the state of any substance, such as from solid to liquid or liquid to gas without a change in temperature. The energy required to convert a substance from the solid to the liquid state is known as the heat of fusion. The energy required to convert one gram of ice to one gram of liquid water at 0 °C is 335J g-1(when expressed on a molar basis, the heat of fusion of water is 7.0 kj mol^{-1} 18 g of water per moles X 335 J g-1). The high heat of fusion of water is attributed to the large amount of energy necessary to overcome the strong intermolecular forces associated with hydrogen bonding.

The density of ice is another important property. At 0 °C the density of ice is less than that of liquid water. Unlike other substances, water reaches its maximum density in the liquid state (near 4°C) rather than as a solid. This is because the water molecules in the liquid state can pack more tightly than in the crystals of ice. Consequently, ice floats on the surface of lakes and ponds rather than sinking to the bottom. This is extremely important to the survival of aquatic organisms of all kinds. Hydrogen bonding also increases the energy required to evaporate water. This is called heat of vaporization. The heat of vaporization of water, or the energy required to convert one mole of liquid water to one mole of water vapor is about 44kJ mol' at 25 ^{0}C. Plants absorb this energy from their surroundings to cause evaporation of water from the surfaces of leaves. As a result, plants undergo substantial heat loss and maintain cooling effects on their leaf surfaces. Such heat loss is an important mechanism for temperature regulation in the leaves of terrestrial plants that are often exposed to intense sunlight.

Water is often called universal solvent because most of the substances will be dissolved than in any other common liquid. This is due to the highly polar character of the water molecule. So that it could partially neutralize the electrical attractions between charged solute molecules or ions by surrounding the ion or molecule called a hydration shell. Water which is present in a hydration shell is often referred to as bound water. It prevents larger molecules, for example, proteins, from approaching close together to form large aggregates and precipitate.

The strong mutual attraction. between water molecules resulting from hydrogen bonding is known as cohesion. Because of cohesion, water contains an exceptionally high surface tension. Due to the cohesive force, which in between water molecules is much stronger than theinteractions between water and air. The result is that the water molecules at the surface are

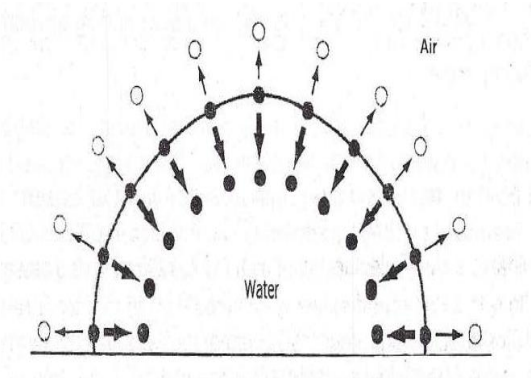

Figure 1.2 Schematic demonstration of surface tension in a water drop. Intermolecular attractions between neighboring water molecules (heavy arrows) are greater than attractions between water and air (light arrows).

constantly pulling into bulk water. Cohesion is also responsible for the unusually high tensile strength of water. It is the maximum tension that an uninterrupted column of any material can withstand without breaking. Although it is a property of metals, under appropriate conditions water columns are also capable of withstanding high tensions on the order of 30megapascals (MPa). The cohesive forces that attract water molecules to each other will also attract water to solid surfaces Known as adhesion. Adhesion is an important factor in the capillary rise of water in small diameter conduits. The combined properties of cohesion, adhesion, and tensile strength help to explain the water rise in capillary tubes and is exceptionally important in maintaining the continuity of water columns in plants.

1.3 WATER TRANSPORT PROCESSES

When water flows from the soil through plants to the atmosphere it travels through mediums such as cell walls, membrane, cytoplasm, and air spaces. The mechanisms of water transport vary with the type of medium. Along with water, substances that move from one region to another is commonly known as translocation. Mechanisms of translocation depending upon the usage of metabolic energy may be classified as either active or passive. In plants translocation of water is clearly a passive process that occurs by one of two physical processes: bulk flow or diffusion. In the case of water, diffusion is called osmosis.

1.3.1 Bulk Flow

Bulk flow or mass flow is a pressure driven process by which molecules of the substance move in a mass. This pressure driven bulk flow of water is the predominant mechanism responsible for long distance transport of water in the plant via xylem.

1.3.2 Diffusion

Diffusion can be interpreted as a direct movement of substances from a region of high concentration to a region of low concentration by the random thermal motion of individual molecules. The rate of diffusion movement is directly proportional to the concentration gradient.

The diffusion coefficient is a characteristic of the substance (larger molecules have smaller diffusion coefficients) and depends on the medium (diffusion in air is much faster than diffusion in a liquid). In case of gaseous diffusion, difference in density is expressed in g m-3

or vapor pressure (KPa, Kilopascal) in place of concentration. The negative sign in the equation indicates that the flux moves down a concentration vapor pressure gradient. The diffusion driven by concentration or vapor pressure differences is a significant factor in the uptake and distribution of water, gases and solutes throughout the plant. Supply of carbon dioxide for photosynthesis and loss of water vapor from the leaves are the two important processes which explain the concept of diffusion.

1.3.3 Osmosis

Movement of a solvent such as water from a region of high free energy to a region of low free energy through a selectively permeable membrane is known as osmosis. Membranes of plant cells are selectively permeable, that is they allow the movement of water and other small uncharged substance across them more readily than the movement of larger solutes and charged substances. Like bulk flow, osmosis occurs spontaneously in response to a driving force. In simple diffusion, substances move down a concentration gradient: in bulk flow substances move down a hydrostatic pressure gradient; in osmosis, both types of gradients influence transport. The direction and rate of water flow across a membrane are determined not solely by the concentration gradient of water or by the pressure gradient, but by the sum of these two driving forces.

This process can easily be demonstrated in the laboratory using a device known as an osmometer. Osmometer is constructed by closing off the open end of a thistle tube with a selectively permeable membrane. To demonstrate osmosis concentrated solution of sucrose is placed in the hollow pore of an inverted thistle tube and pure water is placed in a beaker. The sucrose solution contained thistle tube is then immersed in the beaker of water. Over a period, the volume of solutions in the tube will increase. This increase in volume is due to a net diffusion of water across the membrane into the solution. This occurs because the chemical potential of water in pure water in the beaker is higher than the chemical potential of water in the sucrose solution. As the transport of water proceeds, the height of sucrose solution in the thistle tube increases. Therefore, hydrostatic pressure on the membrane increases. This hydrostatic pressure will tend to press water molecules through the membrane and out of the sucrose solution. When the hydrostatic pressure developed in the tube is sufficient to balance the force driving the water into the solution, further net movement of water through the membrane ceases. Equilibrium with respect to water movement across the membrane has been reached (Figure 1.3). The equilibrium hydrostatic pressure when measured in units of pressure, (force per unit area) is known as osmotic pressure, denoted by the Greek symbol (Ψ_s). It is convention to define osmotic potential as the negative force of the osmotic pressure.

Most of the water in a mature plant cell is present in the vacuoles. This water moves across the plasma membrane and vacuolar membrane (tonoplast) and the layer of protoplasm between these membranes by osmosis. The most important factor to remember is that osmosis is driven not only by the concentration of dissolved solute but also by pressure differences. Both factors influence the overall chemical potential of water, which is the ultimate driving force for water movement in plants.

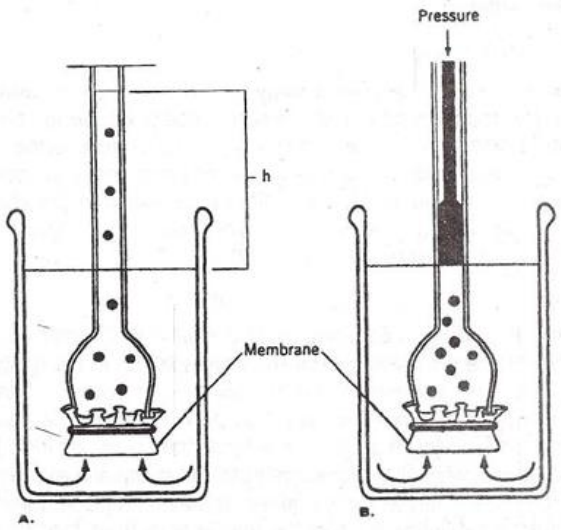

Figure 1.3 A demonstration of osmosis. Initially, water will diffuse across the membrane in response to a water potential gradient. (A) will continue until the force of hydrostatic pressure developed in the tube is sufficient to balance the force driving the water into the solution or (B) the pressure applied by the piston.

1.4 OSMOSIS AND CHEMICAL POTENTIAL

Osmosis is an energetically spontaneous process as it is water movement down a chemical potential gradient, from a region of high chemical potential to a region of low chemical potential. This means that water moves from outside to inside the cell. Net movement of water stops when there is no longer an energy gradient across the membrane. To understand water transport by osmosis, we need to examine more closely what influences the chemical potential of water.

1.4.1 Chemical Potential

The chemical potential is the free energy per mole of any substance in a chemical system. Therefore, the chemical potential of a substance under conditions of constant pressure and temperature depends on the number of moles of substance that is present. In discussing plant water relations, we generally refer to the chemical potential of water as water potential (Ψ_w). Water potential is the difference between the chemical potential of water at any point in a system (μ_w) and that of pure water under standard conditions (μ^0_w)·With the following formula, it can readily be calculated.

$\Psi_w = \mu_w - \mu^0_w = RT \text{ In } e/e^0$

In the formula, R is the gas constant (8.32, J mol:' K-1),T is the absolute temperature (in degrees Kelvin, or OK),e the vapor pressure of the solution in the system at temperature, and e^O the vapor pressure of pure water at the same temperature. The expression of RTIn *(e/eO)* is zero. Knowing this, we can say that pure water has a potential of zero. In biological systems, however *(e/eO)* is generally less than zero, making In*(e/eO)* negative. Consequently, the water potential of biological systems is usually expressed as a negative quantity. Since pure, unconfined water is defined as having a potential of zero, any dilution of water with a solute establishes a potential that is less than that of pure water and is expressed as a negative number. To express the water potentials in pressure units, the above water potential equation is divided by the partial molal volume of water (V_w) which is the volume occupied by one mole of water molecules.

$$\Psi_w = \mu_w - \mu^0_w / V_W = \frac{RT \text{ In } e/e^0}{V_w}$$

Where $\mu w - \mu^0_w$ is the difference between chemical potential of water in solution and that of pure water. The units of above equation are:

$$\frac{\text{erg/ mol}}{\text{cm}^3 / \text{mol}} = \frac{\text{erg}}{\text{cm}^3} = \text{ and 1 bar} = 0.989 \text{ atm} = 10^6 \text{ dynes/cm}^2$$

The concept of water potential is introduced byR.O. Slatyer and SA Taylor in 1960. And it was further simplified as

$$\Psi_w = -\pi + P$$

Where P is the hydrostatic pressure of the solution which may be positive, as in turgid cells, or negative, as in xylem water. The symbol π is called osmotic pressure and is the negative of osmotic potential (Ψ_s).

The water potential of biological systems is determined by choosing the pure water as a reference state atmospheric pressure. Under these conditions there is neither hydrostatic pressure nor dissolved solutes; that is both P and π are zero. So, the value of Ψ_w for pure water according to the above equation is zero. This is not to say that the chemical potential of pure water is zero. The value of $\mu°_w$ is in fact very high but μ_w for pure water is zero.

1.5 THE COMPONENTS OF WATER POTENTIAL

Water potential of solution may be also defined as the sum of its component potentials, such as solute potential *(Ψ_s)* and pressure potential (Ψp).

$$\Psi_w = \Psi_s + \Psi_p$$

Solute potential (Ψ_s) The decrease in water potential brought about by dissolved solutes in solution is called solute potential or osmotic potential. Solutes reduce the free energy of water by diluting the mole fraction of water. Solute potential in a solution depends on the total number of solute particles in a solution rather than on their kind or their change. For ionic solutes that dissociate into two or more particles, the solute concentration of the solution must be multiplied by the number of dissociated particles. Thus, for example, if we dissolve 0.1 mol of sucrose (non-dissociating substance) in 1L of water, we obtain a solution with an osmolarity of 0.1 mol L-1 (solute concentration of the solution is expressed as osmolarity: moles of total dissolved solutes per liter of water). If we dissolve ionic substance, for example, sodium chloride in liters of water, the resulting solution has an osmolarity of 0.2 mol L^{-1} because NaCl dissociates into $Na^+$and CI^- particles.

• At 20°C (293K) the solute potential of 0.2 mol NaCI (-0.244 MPa) and 0.33 mol CaCl solutions is equal to solute potential of 0.1 mol sucrose solution (-0.488 MPa). In a solution that consists of several different solutes the solute potential is the sum of the individual solute potentials contributed by each of the solutes.

Pressure *(Ψ_p)*

The term Ψ_p is the hydrostatic pressure of the solution. Sometimes Ψ_p is called pressure potential. Positive pressures raise the water potential whereas negative pressures reduce it.

ln a laboratory osmometer, this pressure *(Ψ_p)* can be estimated as the difference between atmospheric pressure (0.1 MPa) and the hydrostatic pressure generated by the height of the water column. In cells, this pressure component arises from the force exerted

outwardly against the cell walls by the expanding protoplast. This is known as turgor pressure. An equal but opposite inward pressure called wall pressure is exerted by the cell wall. A cell experiencing turgor pressure is said to be turgid. A cell that experiences water loss to the point where turgor pressure is reduced to zero is said to be flaccid. The value of Ψ_p may also be negative, for example, in the xylem and in the walls between cells, where a tension or negative hydrostatic pressure can develop.

As water passes through the membrane by osmosis and into a cell, it often encounters resistance from other substances, a factor that contributes to the matric potential(Ψm)' It may be defined as the energy lost (with respect to pure water) as water diffuses and interacts with other substances in the diffusion medium. Since the Ψ_m is not applicable and difficult to measure in osmotic systems, we consider it to be negligible when solving problems of osmosis in plant cells. The osmotic potential of most plant cells is due to the contents of the large central vacuole. Except meristematic cells and highly specialized cells, majority of the cell vacuoles contain 50-80% of the cellular water and dissolved solutes including sugars, organic salts, organic acids and anthocyanin pigments. Most of the remaining cellular water is in the cell wall spaces, while the cytoplasm accounts for around 5-10 percent. The osmotic potential of parenchyma cells is typically in the range of -0.1 to -0.3 MPa.

1.6 Water Potential Gradient

When dealing with water transport at the cell level, the water potential is significantly influenced by two components such as dissolved solutes and hydrostatic pressure.

$$\Psi_w = \Psi_s + \Psi_p$$

The concept of water potential can be fully explained through the following examples. First. imagine an open beaker full of pure water at 20 °C (Figure 1.4 A). Since the water is open to the atmosphere, the hydrostatic pressure of the water is the same as atmospheric pressure (Ψp= O MPa). There are no solutes in the water, so Ψ_s= OMPa, therefore, the water potential of water in an open beaker is OMPa ($\Psi_w = \Psi_s + \Psi_p$). Now imagine dissolving sucrose in the water to a concentration of 0,1 M (Figure 1.4 B).This addition lowers the osmotic potential (Ψ_s) to - 0.244 MPa and decreases the water potential (Ψ_w)to - 0.244 MPa .

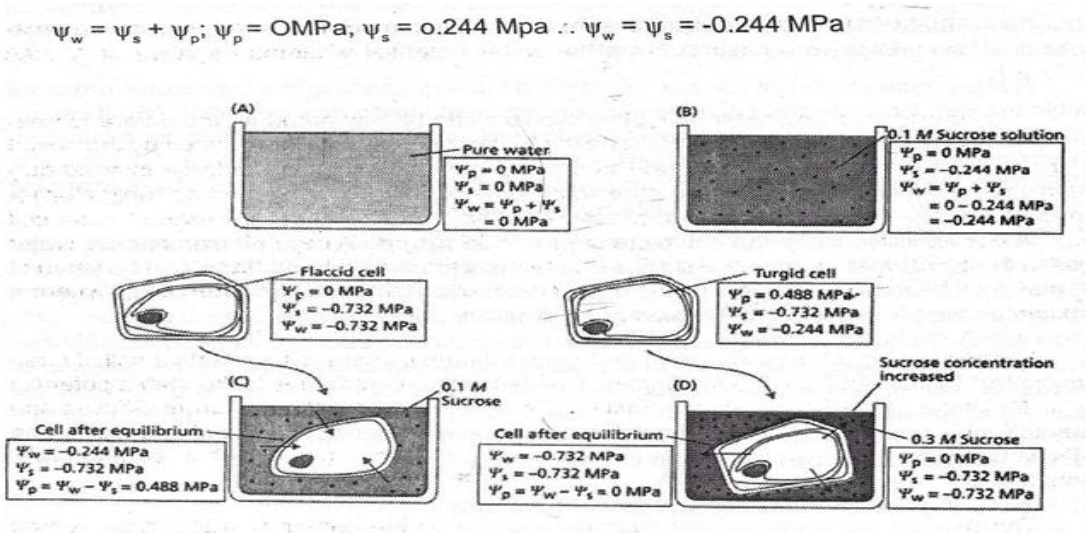

Figure 1.4 Examples illustrating the concept of water potential and its components. (A) Pure water, (B) A solution containing 0.1M sucrose. (C) After a flaccid cell is dropped in the 0.1 M. sucrose solution. (D) After a turgid cell is placed in the 0.3 M sucrose solution.

Next consider a flaccid plant cell (a cell with no turgor) that has a total internal solute concentration of 0.3 M (figure 1.4 C). This solute concentration gives an osmotic potential(Ψ_s) of -0.732 MPa, Because the cell is flaccid, the internal pressure is zero. So here also the water potential is equal to osmotic potential, which is -0.732 MPa. What happens if this cell is placed in the beaker containingO·.1 M sucrose (see; Figure 1.4 C). Water will move from the sucrose solution to the cell because the water potential of the sucrose solution (-0.244 MPa) is greater than the water potential of cell (-0.732 MPa). That is water moves down a water potential gradient. As water moves into the cell, the hydrostatic pressure or turgor pressure (Ψ_p) of the Cell increases. Consequently, the cell water potential (Ψ_w) increases and the difference between inside and outside water potential ($\Delta\Psi_w$)'is reduced. Eventually, cell Ψ_p increases enough to raise the cell water potential to the same. Value as the Ψ_w of the sucrose solution. At this point equilibrium is reached (Ψ_w = OMPa), and net water transport ceases.

In fact, the tiny amount of water taken up by these does not significantly affect the solute concentration of the sucrose solution because the volume of sucrose solution is very much higher than that of the cell. Hence osmotic potential (Ψs) hydrostatic pressure (Ψp) and water potential (Ψw) of the sucrose solution are not altered. Therefore, at equilibrium that water potential of the cell sap is equal to the water potential of sucrose solution in the beaker, which is -0.244 MPa.

A slight increase in cell volume increases a large increase in the hydrostatic pressure within the cell, because plant cells are surrounded by relatively rigid cell walls. So, it can be assumed that a tiny amount of water taken up by the cell does not affect its solute concentration. It remains unchanged during equilibrium process and that solute potential (Ψ_s) remains at -0.732 Mpa. With these assumptions, we can obtain cell hydrostatic pressure by rearranging equation $\Psi_p = \Psi_w - \Psi_s$ = (-0.244) - (-0.732) = 0.488 MPa.

Water can also leave the cell by osmosis. If in the previous example, when a turgid plant cell from 0.1 M sucrose solution is removed and placed in a 0.3M sucrose solution(Figure 1.4 D), water will move from the cell to the solution because the water potential of the0.3 M sucrose solution (-0.732 MPa) is more negative than the water potential of the cell (-0.244 MPa). As water leaves the cell, the cell volume decreases i.e. its protoplast shrinks away from the cell wall. This condition of a cell is known as plasmolysis. As the cell volume decreases, cell Ψ_p andΨ_w also decrease until water potential of the cell is equal to water potential of the solution which is -0.732 Mpa. From the water potential equation, we can calculate that at equilibrium Ψ_p = 0 MPa. As before we assumed that the change in cell volume is small, so we can ignore the changes in Ψ_s .

1.7 MEASURING WATER POTENTIAL AND ITS COMPONENTS

Over the years, very simple and accurate methods have been developed by plant physiologists for assessing water potential and its components in plants because the growth, metabolic activities and productivity is influenced markedly by water. Tissue weight Change Method Water potential of tissues can be estimated simply by equilibrating pre weighed samples of tissue in solution of known osmotic potential. The main objective of this method is to determine the solution with an osmotic potential equivalent to the water potential based on the change in the weight of the tissue.

- If the osmotic potential of the bathing solution is more negative than the water potential of the tissue, water will leave the tissue, as a result the tissue lose weight.

- If the osmotic potential of the bathing solution is less negative than the water potential of the tissue, the tissue will take up water and gain weight.
- The solution at which the tissue neither gains nor loses weight is deemed to have an osmotic potential equivalent to the water potential of the tissue.
- Samples of uniform size are prepared, weighted. and placed in solutions of known molality (0.1,0.2,0.3,0.4) of sorbitol or Mannitol (or) Polyethylene glycol. After allowing 30minutes for the tissue and bathing solution to come to equilibrium, the tissue is blotted to remove excess solution and weighed once again, the weight gain or loss is then calculated as a percentage of the original weight and plotted against the concentration of the solution.
- Water potential of the plant tissues is also measured using Thermocouple Psychrometry.
- Osmotic potentials are estimated in leaf epidermal cells and other cells by observing incipient Plasmolysis.
- Negative hydrostatic pressures normally present in xylem vessels can be measured with pressure bomb.

1.8 THE IMPORTANCE OF WATER POTENTIAL

The concept of water potential has two principal uses. First, it is the quantity that ‘governs transport across cell membranes. Second, water potential is used as a diagnostic tool by the plant scientists to assign a precise value to the water status in plant cells and tissues. The lower the water potential in a cell or tissue, the greater its ability is to absorb water. Conversely, the higher the water potential, the greater the ability of the tissue to supply water to other more desiccated cells and tissues. Thus, water potential is used to measure water deficit and water stress in plant cells and tissues. Figure 1.5 shows some of the physiological changes that plants experience as they become dry under conditions when the transportational water loss to the atmosphere 'exceeds the water absorption from the soil. The process that is most affected by water deficits cell growth. More severe water stress leads to inhibition of cell division, inhibition of wall and protein synthesis, accumulation of solutes, closing of stomata and inhibition of photosynthesis.

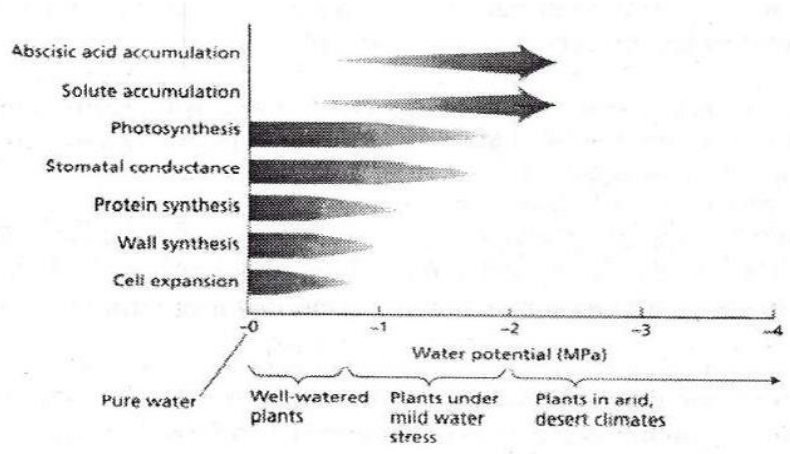

Figure 1.5 Water potential of plants under various growing conditions, and sensitivity of various physiological processes to water potential. The intensity of bar color corresponds to the magnitude of the process. For example, cell expansion decreases as water potential falls.

Generally, leaves of most plants rooted in wall watered soils are likely to have water potential between about -0.2 and -0.8 Mpa. With decreasing soil moisture supply, leaf water potential will become more negative than -0.8 MPa and leaf growth rates will decline. Most p\ant tissues stop growth completely (i.e. will not enlarge) when water-potential drops to about - 1.5 MPa. Leaves of most herbaceous plants usually do not recover if the water potential drops below about -2 to -3 MPa. In contrast, leaves of desert shrubs have a greater ability to survive under conditions of severe drought, perhaps in the range of - 3 MPa to - 6 MPa. Viable, air-dried seeds are also likely to have very low water potential perhaps as low as - 0. 6 MPa to - 10MPa or even much lower depending on the extent of dried and the plant species.

1.9 SUMMARY

Water is the main constituent of living organisms. It possesses certain unique physicochemical properties that enable water to play many important roles in the physiology of plants. The thermal properties of water contribute to regulation of temperature there by ensuring that plants do not cool down or heat up too rapidly. The solvent properties of water facilitate appropriate medium for the uptake and distribution of mineral nutrients and other solutes required for growth. The transparency of water permits light to penetrate the aqueous medium of cells and used to perform photosynthesis or control development. Hydrogen bonding is largely responsible for the main unique properties of water. The most important property of water is that it is a liquid over the range of temperatures most compatible with life. The combination of high specific heat and thermal conductivity enables water to absorb and redistribute large amounts of heat energy without correspondingly large increase in temperature. Unlike other substances, water reaches its maximum density in liquid state that is extremely important for the survival of aquatic organisms of all kinds.

The movement of substances along with water in the plant is commonly referred to as translocation. The direct movement of substances from a region of high concentration to region of lower concentration is called diffusion. Overall Fick's law explains that the rate of diffusion is directly proportional to the cross-sectional area of the diffusion path and to the concentration or vapor pressure gradient, and it is inversely proportional to the length of the diffusion path. At the cellular level, water moves primarily by osmosis, in response to a chemical potential gradient across a selectively permeable membrane. Water movement is primarily a function of the difference in chemical potential between the water in the cell and the water in the cell environment. The chemical potential of water in plant water relations can be expressed as water potential, defined as the sum of two easily measured quantities: hydrostatic pressure and osmotic pressure.

In view of the significance of water that influences growth & metabolic activities and productivity, many methods and mechanisms have been developed by plant physiologists for assessing water potential like tissue weight change method, Thermocouple psychrometry, Incipient plasmolysis and Pressure bomb.

1.10 MODEL QUESTIONS

1. Give an account of thermodynamics of plant water relations. Define osmotic and water potential. Suggest simple method to determine water potential.
2. Write short notes
   a) Significance of water potential

b) Hydrogen bonding and its contribution to the unique properties of water.

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Dr. C.V.S Bhaskar

LESSON - 2

WATER TRANSPORT THROUGH XYLEM

OBJECTIVE:

In this lesson the upward movement of water from ground level to the top of the tallest trees were described by a variety of mechanisms including root pressure, capillarity and cohesion-tension theory.

STRUCTURE OF THE LESSON:

2.1 INTRODUCTION

Movement of the absorbed water through the vascular system from the xylem terminal in the root to those in the leaf is called ascent of sap. Sometimes, water transport covers more than 110 meters against a gravitational bull as in the case of a Californian Redwoods *(Sequoia sempervirens)* and an Australian *Eucalyptus regans*(130 meters). The problem that has long held plant physiologists is the mechanism by which water moves to the top of the tallest tress movement. The forces required to move water to such heights are substantial. One atmospheric pressure supports a column of water 10.3 m or a column of mercury 760 mm in height. To push water from the ground level to the top of the tallest tree requires a top to bottom pressure difference of about 1.0 to 1.5 MPa. So, it is evident that water is not pushed to the top of tall trees by atmospheric pressure. Even a force of 1.0 to 1.5 MPa would not be sufficient to move water up the tallest tree. Water moving through the plant will encounter a certain amount of resistance due to irregular wall surfaces and perforation plates after conducting tracheid and vessels. In addition to this frictional resistance, we must consider gravitation force. The weight of a standing column of water 100 m tall creates a pressure of 1 MPa at the bottom of the water column. This pressure gradient due to gravity must be added to that required to cause water movement through the xylem. Thus, we calculate that a pressure difference of about 2.0 to 3.0 MPa would be required to move water from ground level to the top of the tallest trees. How can such pressure be generated? To answer this question, from time to time various theories have been proposed, the prominent beings are root pressure, capillarity and cohesion - tension theory. Before we are going to discuss these theories in some detail, we must consider anatomy of water conducting cells.

2.2 WATER TRANSPORT BY TRACHEID AND VESSELS

One of the distinguishing features of vascular plants is the presence of vascular tissues, the xylem and phloem, which conduct water and nutrients between the various organs. Xylem

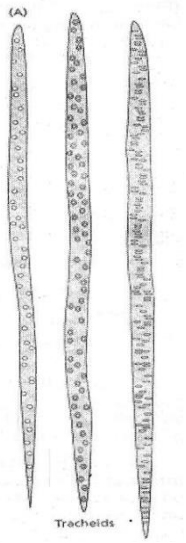


Figure 2.1 The shape of plant tracheids. The walls contain numerous bordered pits.

tissue is responsible for the transport of water, dissolved minerals, and on occasion, small organic molecules from the root to the aerial organs through the stem. Phloem, on the other hand, is responsible for the translocation of organic substances from sites of synthesis to storage sites or sites of metabolic demand. Xylem consists of fibers, parenchyma cells, and tracheary elements. Fibers are very elongated cells with thickened secondary walls, which provide structural support for the plant Parenchyma cells of the xylem serve as storage as well as the lateral translocation of solutes. Tracheids and vessel elements together known as tracheary elements. Tracheids are present in both angiosperms and gymnosperms. Vessel elements are found only in angiosperms and a small group of gymnosperms called the Gnetales.

When mature, the trachieds and vessels form an interconnected network of nonliving cells devoid of all protoplasm. Tubular and hollow nature of trachieds and vessel elements with their extensive interconnections enables them to transport large volumes of water throughout the plant with great efficiency. Tracheids are elongated, spindle shaped cells with diameters in the range of 10-50 urn, (Figure 2.1) They generally measureless than 1 μm in length. The cell walls of trachieds are composed of cellulose, hemicellulose and lignin. Because of the high lignin content, secondary walls of tracheids are less permeable to water than are the primary walls of growing cells. On the other hand, the secondary walls provide additional strength and help to prevent the cells from collapsing under extreme negative pressure developed in the actively transpiring plants. Though trachieds conduct water, the thickened secondary walls also provide structural support to the plant.

The movement of water between trachieds is facilitated by small interruptions, known as bordered pits in the secondary wall. Bordered pits do not deposit secondary wall material during the development of trachieds, Hence, they have only the middle lamella and primary walls to separate the hollow core or lumen of the cell (Figure 2.2). Pits of one tracheid are typically located opposite pits of adjoining tracheid, forming pit pairs. The combined middle lamella and primary walls between pit pairs is known as the pit membrane and having around

0.3 µm diameter openings that permit the relatively free passage of water and solutes. Bordered pit pairs have secondary wall projections over the pit area. The pit membrane in tracheids of gymnosperms usually have a central thickening called a torus(Figure 2.2). The torus acts like a valve to close the pit by lodging itself in the circular or oval wall thickenings bordering these pits. When pressure is unequal in adjacent vascular elements, the torus drawn toward the element with the lower pressure and seals off the pit.

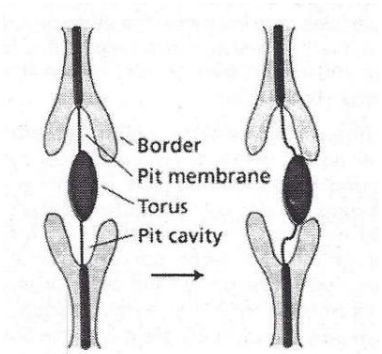


Figure 2.2 Diagram of a bordered pit with a torus either cantered in the pit cavity or lodged to one side of the cavity, thereby blocking water flow.

Successive trachieds overlap at their tapered ends. As a result, they line up in files running longitudinally throughout the plant. Water moves between adjacent tracheids either vertically or longitudinally through the pit pairs by their overlapped regions. The movement of water is facilitated by the openings in the pit membranes. Vessels are very long tracheary elements made up of individual units, known as vessel members. (Figure 2.3) They are arranged end to end in longitudinal series to form a larger unit called a vessel. At maturity, the end walls of the vessel members are dissolved away and form perforation plates. If the perforations are elongate and parallel they are called scalariform. If they are net like patterns they are known as reticulate. These perforations allow free flow of water between successive vessel members. However, absence of perforation plates at the ends of vessels, that is, the last vessel member in a sequence, facilitates lateral conduction of water from one vessel to the next due to the presence of pit pairs.

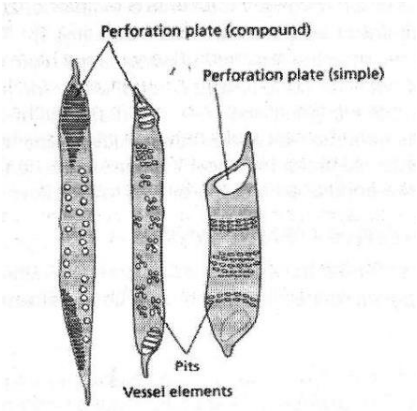


Figure 2.3 Vessel elements with perforation plates and pits.

The exact length of vessels is difficult to ascertain. It is highly variable. in maples, vessels range from 40-60 µm in diameter. Whereas in some Quercus species vessels range from 300-500 µm in diameter. Similarly, length of the vessels in maple account for 4 cm or less, but in some cases, they may reach 30 cm to 30 m length *(Quercus).* Because of extensive branching of the vascular system and the large number of lateral connections between the overlapping

tracheary elements, the xylem constitutes a single continuous, interconnected system of water conducting conduits between the extremes of the plant from the tip of the longest root to the outermost margins of the highest leaf.

Vessels are more advanced than trachieds. Gymnosperms entirely consist of tracheids with their xylem. Compared to gymnosperms, angiosperms do possess trachieds, but the bulk of the water is conducted through vessels efficiently because vessels are larger in size.

Jean L.M.Poiseuille, a French scientist, developed an empirical equation to show the relationship between flow rate and size of conduits.

$$Jv = \Delta P\ \pi r4/8\ ή$$

When a fluid is pressure driven, the volume flow rate (Jv) is a function of the viscosity of the liquid (ή) the difference in pressure or pressure drops(ΔP) and the radius of the conduit(r).The above equation can be applied to movement of water in the xylem, because water movement in the xylem is driven by a difference in pressure between the soil and the leaves. The key point to remember is that the volume flow rate is directly proportional to the fourth power of the radius. The volume flow rate through a vessel with a dimeter of 200 μm is 5 times that of the tracheid (40 μm). The high rate of flow in the larger vessels occurs because the flow rate of water is not uniform across the conduit. The flow rate of molecules near the conduit walls is reduced by friction, due to adhesive- forces between the water and the conduit wall. As the diameter of the conduit increases, the proportion of molecules near the wall and consequently subject to these frictional forces will decrease. In other words, the faster moving molecules in the center of the conduit constitute a larger proportion of the population and the overall rate of fl9w increases accordingly.

2.3 THE MECHANISM OF ASCENT OF XYLEM WATER:

The mechanism of water transport in plants can be explained by three prominent theories such as root pressure, capillarity and cohesion - tension theory.

2.3.1 Root Pressure

Plants sometimes exhibit a phenomenon referred to as root pressure or positive hydrostatic pressure. For example, if the stem of a well-watered herbaceous plant is cutoff above the soil line, the stump will often exude sap from the cut xylem for many hours. Ifa manometer is sealed over the cut stump; positive pressures can be recorded. This pressure is known as **root pressure** because the forces which give rise to exudation originate in the root. These pressures can be as high as 0.05 to 0.5 MPa.\

Root pressure can be understood as positive hydrostatic pressure in the xylem. It has its basis in the structure of roots and the active uptake of mineral salts from the soil. The xylem elements are in the central core of a root, called the stele, which in turn is surrounded by a layer of endodermis. Water can move into or out of the stele only by first passing through the membranes of the endodermis and then through the plasmodesmata connections. This is because of the Casparian band, which is present in the radial and transverse walls of the endodermal cells, prevents the movement of water through the apoplastic space of the endodermis.

A large number of anatomical and physiological studies have established that the region of most active water uptake lies near the root tip. Beyond this generalization, the permeability of roots to

water varies widely with age, physiological condition, and the water status of the plant. Studies with young roots have shown that the region most active with respect to water uptake starts about 0.5 cm from the tip and may extend down the root as far as 10 cm . Little water is absorbed in the meristematic zone itself, presumably because the protoplasm in this zone is dense and there are no differentiated vascular elements to carry the water away. The region over which water appears to be taken up most rapidly corresponds generally with the zone of cell maturation. This is the region where vascular tissue,in particular the xylem, has begun to differentiate. Also in this region the deposition of suberin and lignin in the walls of endodermal cells is only beginning and has not Suberization and lignification reduce permeability Suberization and lignification of endodermis beginning Stele Cortex Xylem differentiating Region of elongation Meristematic region Root cap Most rapid entrance of water and salt Slow entrance of water and salt Root hair zone Relatively impermeable to water Slow entrance of water and salt because of decreasing permeability

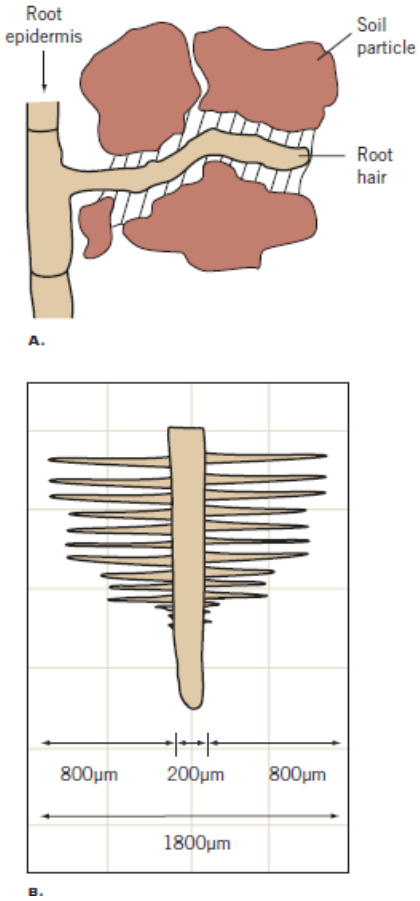


FIGURE 2.4 Diagrammatic illustration of the relationshipbetween differentiation of root tissues and water uptake.

yet reached the point of offering significant resistance to water movement.The region of most rapid water uptake also coincides with the region of active root hair development.Root hairs are thin-walled outgrowths of epidermal cells that increase the absorptive surface area and extend the absorptive capacity into larger volumes of soil(Figure 2.4). Depending on the species and environmental conditions, root hairs may reach lengths of 0.1mm to 10mm and an average diameter of 10 µm. In some species such as peanut, pecan, and certain conifers, root hairs are rare or absent. More commonly a single root tip may contain as many as 2,500 hairs cm−2 and may increase the absorbing surface of the root 1.5 to twentyfold. For two reasons, root hairs greatly increase the contact of the root with soil water. First, their small diameter permits root hairs to penetrate capillary spaces not accessible to the root itself (Figure 2.4). Second, root hairs extend contact into a cylinder of soil whose diameter is twice that of the length of the hair

Further, the roots absorb ions from the dilute soil solution and transport them into the xylem. The active buildup of solutes in the xylem sap leads to a decrease in the xylem osmotic potential (Ψ_s) and thus a decrease in the xylem water potential (Ψ_w). In response to the lowered water potential, water moves from the surrounding cortical cells into the xylem through the membranes of the endodermal cells. Since the Casparian band prevents the free return of water to the cortex, positive hydrostatic pressure is developed in the xylem. In effect the whole root acts like an osmotic cell in which the endodermis behaves as differentially permeable membrane, the ions accumulated in the xylem represent the dissolved solute. The question to be answered at this point is whether root pressure can account for the rise of sap in a tree. For several reasons, the answer to this question is no.

The measured root pressure values are only in the range of s0.05 to 0.5 MPa, which are no more than 16 percent of that required to move water to the top of the tallest trees. In addition, root pressure has not been detected in all species and is not always detectable even those species which do exhibit. Root pressure is most prominent in well-hydrated plants under high humidity where there is little transpiration. Under drier conditions, when transpiration rates are high, water is taken up so rapidly into the leaves and lost to the atmosphere, the xylem is present under tension i.e negative pressure. So, root pressure clearly cannot serve as the mechanism for the ascent sap in all cases. Plants that develop root pressure frequently exhibit exudation of liquid from the leaves, a phenomenon known as **guttation**. Positive xylem pressure causes exudation of xylem

sap through structures called **hydathodes**. They are located near tracheid terminals of the bundle ends around the margins of leaves. The 'dewdrops' that can be seen on the tips of grass leaves in the morning are guttation droplets exuded from such specialized pores.

2.3.2. Water rise by capillarity

The movement of water small distance up a glass capillary tube is called capillarity. When a glass capillary tube is inserted into a volume of water, water will rise in the tube to some level above the surface of the surrounding water. This upward movement of water in such a small diameter tube is due to the interaction of several forces. These include adhesion between water and polar groups along the capillary wall, surface tension which tends to minimize surface area, and the force of gravity acting on the water column. Adhesive forces attract water molecules to polar groups along the surface of the tube. When the water to wall forces is strong, water flows upward along the wall. As water flows upward along the wall, strong cohesive forces between the water molecules act to pull the bulk water up the lumen of the tube. This rise will continue until these lifting forces are balanced by the downward force of gravity acting on the water column. The rise of water in a capillary tube is inversely proportional to the radius of the tube. The smaller the tube, the higher the capillary rise which may be calculated by the following formula:

$$\text{Capillary rise} = \frac{7.9 \times 10^{-6}\ m^2}{\text{Radius}}$$

Where both capillary rise and radius are expressed in meters. For a xylem vessel with 25 mm radius, the capillary rise is about 0.6 m. This rise is only 0.08 mm for a vessel having longer radius of 200 µm. Based on these numbers, capillarity in tracheid and small vessels might account for the rise of xylem sap in small plants to a height of less than0.75m. The capillarity mechanism, therefore, is inadequate to explain the water movement in the tallest tress.

2.3.3 Cohesion tension theory

Henry H. Dixon, an Irish botanist, and John Joley, a physicist in 1894 developed the idea of cohesion-tension mechanism of ascent of sap. The work was published in book form by Dixon with a great wealth of experimental details in 197. The theory states that transpiration pull or tension, cohesion property of water and adhesion between water and cell walls are collectively responsible for the upward movement of water in the tallest trees.

Water movement through the xylem is a bulk f1ow caused by pressure difference ($\Delta\Psi p$) between the roots and the treetops. A negative pressure or tension created due to transpiration is transmitted through the continuous water column within the xylem to the root. The breaking of the water column is prevented by cohesive forces between adjacent molecules of water and adhesion between water molecules and cell walls. Therefore, the cohesion theory for the ascent of sap has three basic components, the driving force, the cohesion of water and the hydration of cell walls (adhesion).

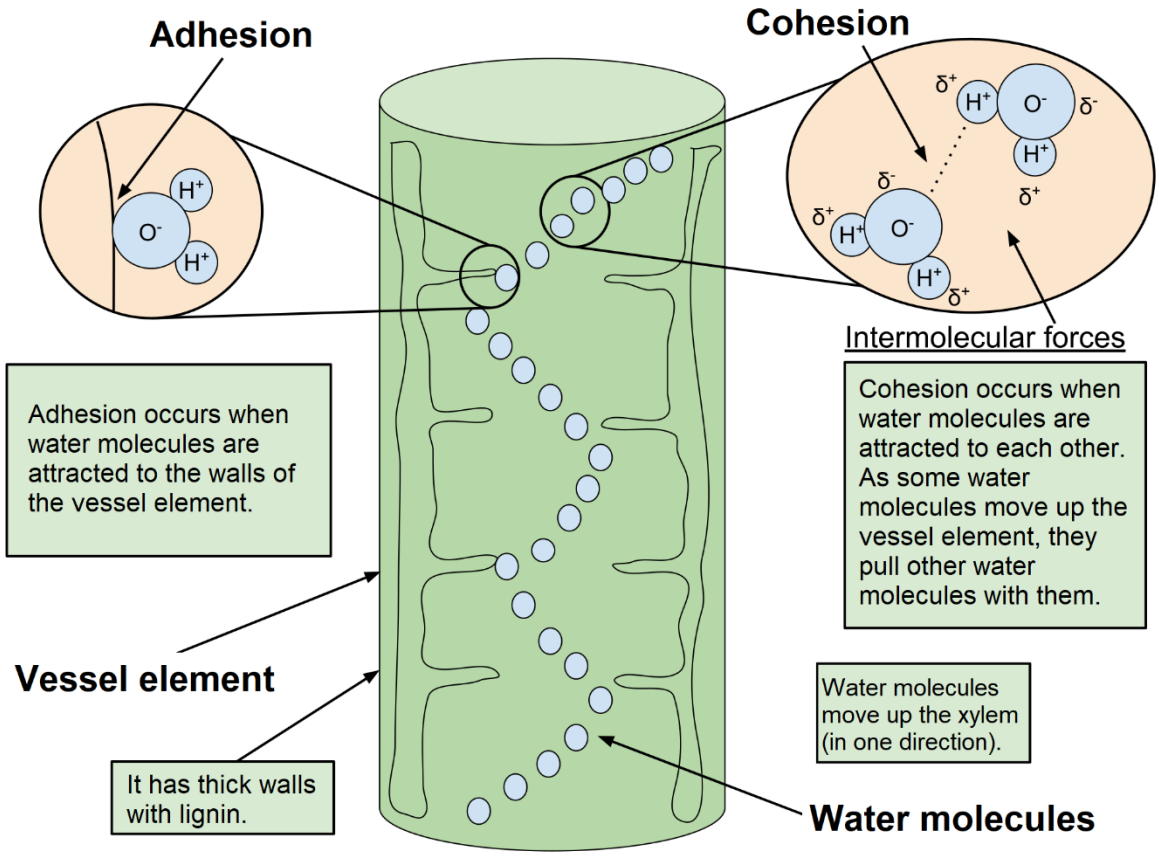


Figure :2.5 The cohesion and adhesion of water in the vessel element helps water move up the vessel without breaking under tension. Adhesion occurs when water molecules are attracted to the walls of the vessel element, which has thick walls with lignin, a stiff substance. Cohesion occurs when water molecules are attracted to each other. This is due to hydrogen bonds, which form between the partially negative oxygen of one molecule and the partially positive hydrogen of another molecule. Hydrogen bonds are a strong intermolecular force. As some water molecules move up the vessel element, they pull other water molecules with them. Water molecules move up the xylem (in one direction). Image modified from FeltyRacketeer6 (CC-BY-SA).

The driving force

The driving force is the gradient in decreasing water potentials from the soil through the plant: to the liquid - air interface at the evaporating surfaces within the leaf. Water covers the surfaces of the leaf mesophyll cells as a thin film. As water evaporates from this thin film, the air - liquid interface retreats into the small spaces between cellulose microfibrils and the angular junctions between adjacent cells. This creates very small, curved surfaces (Figure2.5). As the radii of these curved surfaces progressively decreases, surface tension at the air-water. interface generates an increasingly negative pressure, which in turn tends to draw more liquid water toward the surface. That isas water evaporates from the leaf mesophyll cells it causes a decrease in water in water potential*(Ψ_w)* of those cells in direct contact with the air spaces of the leaf. Due to the negative water potential of the surface cells, water moves into them from the deeper cells of the leaf. To equate water potential value, the leaf cells ultimately tend to draw water from the veins of the leaf, thus subjecting the water in the xylem a state of tension or negative pressure. Because the water column is continuous, this negative pressure or tension is transmitted through the column all the way to the soil. This top to bottom pressure difference ($\Delta\Psi p$) developed between the roots and the treetops via the stem is called the driving force. This driving force drives the upward movement of water in plants-from the roots to the surface of mesophyll cells in the leaf.

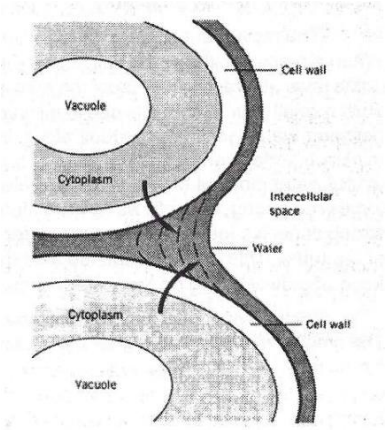
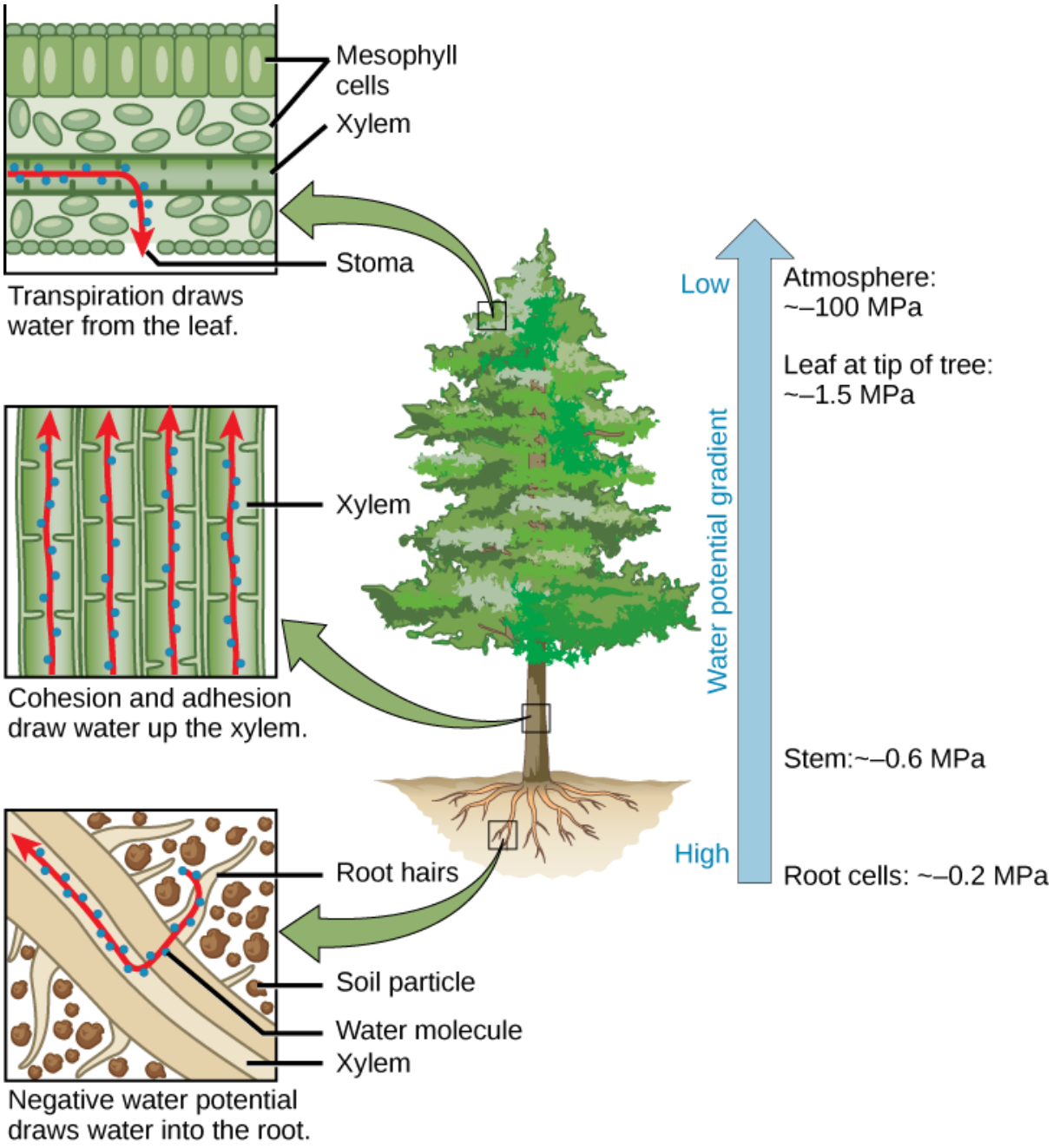


Fig 2.6: Tension (negative pressure) in the water column. Evaporation into the leaf spaces causes the water-air interface (dashed lines) to retreat into the spaces between and at the junction of leaf mesophyll cells, as the water retreats, the resulting surface tension pulls water from the adjacent cells, because the water column is continuous. This tension is transmitted through the column. ultimately to the roots and soil water.

Cohesion theory raises two very important questions.

1. Is the xylem sap of a rapidly transpiring plant under tension?
2. How is the integrity of very tall water columns maintained?

Several lines of evidence made it clear that xylem water is under significant tension. When the xylem of a rapidly transpiring plant is served, it is sometimes possible to hear air being drawn rapidly into the wound. If served beneath the surface of a dye solution, the dye will be rapidly taken up into tracheary elements in the immediate vicinity of the wound. Kramer and Kozlowski (1960) by using Dendrography observed a decrease in diameter of woody stems during excessive transpiration period. This will happen because the stem is slightly elastic and the tension in the water column pulls the tracheary walls inward. In the evening, when transpiration declines, the tension is released and stem diameter recovers.



In a pressure bomb technique adopted by Scholender and co-workers (1965), shoots excised and sealed in a pressure chamber with only the cut surface of the stem exposed. If the water column in the xylem is under tension, as it normally is in a transpiring shoot, it will withdraw from the surface when the stem is cut. Pressuring the chamber will force the water back to the surface. The pressure required to bring the water back to the surface is considered of equal magnitude but of opposite sign to the tension that existed in the xylem prior to excision. With such a device, scholander and others have measured tensions on the order of -0.5 to -2.5 MPa in rapidly transpiring temperate zone trees. The weight of evidence, therefore, clearly supports the hypothesis that the xylem water column is literally pulled up the tree in response to transpiration.

The cohesion of water

The integrity of tall water columns in the xylem depends on tensile strength of the water. Tensile strength is a measure of the maximum tension a material can withstand before breaking. The water molecules have strong mutual attraction (cohesion) due to which they cannot be easily separated from one another (high tensile strength). A variety of factors including the diameter of conduit, the properties of conduit walls, and presence of any dissolved gases or solute will influx the tensile strength of water. It has been estimated that pure water, free of dissolved gases, is able to withstand tensions as low as -25 to -30MPa at 20 ^{0}C. This is approximately 10 times greater than the -2.5 to -3.0 MPa required to pull an uninterrupted water column to the top of the tallest trees.

Adhesion

Cell wall polysaccharides have a great affinity for water molecules. Wall surfaces usually have a net negative charge that attracts the slightly positive sides of the polar water molecules. This is called hydration. The wall surface which can bind with water is called matrix. Water can be held by hydrophilic wall surfaces with tension in the order of -100 MPa to -300 MPa. Gravity, that is the weight of water column in the xylem vessel, cannot remove water against such powerful forces. There is only one relevant but refutable objection to cohesion tension theory. Xylem water contains several dissolved gases such as CO2, 02, and nitrogen. When the water column is under tension, there is a tendency of these gases to come out of solution. As a result, submicroscopic bubbles first form at the interface between the water and the walls of xylem elements, probably in small, hydrophobic crevices or pores in the walls. This phenomenon is sometimes called 'air seeding'. These small bubbles may redissolve, or they may coalesce and expand within the water column of a conduit. This phenomenon of bubble formation is known

as cavitation. The resulting gas bubble forms an obstruction, called embolism, (Gr. Embolus= Stopper), in the conduit. Cavitation embolism of the xylem breaks the continuity of the water column and stops water transport. However, the impact of xylem cavitation on the plant is minimized by several means. Tracheids and vessels constitute multiple, parallel, interconnected pathways for water movement. When cavitation blocks water movement within the cavitated vessel, it does not completely stop water movement in the cell file. Because tracheary elements are interconnected through wall pits, water can detour around the blocked vessel by moving through adjacent tracheary elements (Figure:2.5).

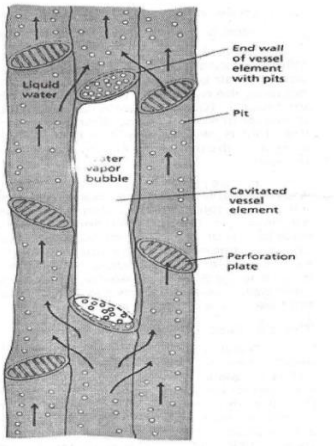


Figure 2.5 Water detours around activated vessel element. Water can detour around the blocked vessel by moving through adjacent tracheary elements.

The spread of the vapor bubble throughout the xylem is eventually stopped by an end wall that lacks a perforation plate. Gas bubbles may also be eliminated from the xylem. At night, when transpiration throughout. Xylem Ψ_w increases and the water vapor and gases may simply dissolve back into the solution of the xylem.

So, from the above discussion, the cohesion tension theory is now the available mechanism to explain the mechanism of ascent of sap in the tallest trees and it has got the following essential features.

1. Water inside the xylem forms a continuous column from top to bottom.
2. Water evaporates from the mesophyll cell surfaces (transpiration), due to which driving force or pulling force Develops putting the water column inside the xylem under tension.,
3. The tension may cause a break in the water column but due to the cohesive and adhesive property of water the continuous column does not break.

2.4 SUMMARY

In plants, water is mainly transported through an interconnected system of open conduits formed as series of tracheary elements. Xylem and phloem transports water and mineral nutrients between various organs. Translocation of water in the xylem is facilitated by components like fibers, parenchyma cells, tracheary elements. The phenomenon of water absorption and transport to all the aerial parts is very common process. But the question, how the integrity of the xylem water column is maintained and how it moves to the top of the tallest trees, has attracted the attention of many plant physiologists. In this connection several mechanisms have been proposed, but the only one to have stood-the test of time combines transpiration with the strong cohesive forces of water.

H.H. Dixon proposed the cohesion theory in 191·4 that gave a detailed account of the movement of water through the plant Although aspects of the cohesion tension theory of sap ascent are intermittently debated, an overwhelming body of evidence supports the idea that water transport in the xylem is driven by pressure gradient. High transpiration generates negative pressure in the xylem water may cause cavitation (embolisms) in the xylem' But the structure of tracheid or vessel members play a major role for minimizing the effect of embolism.

2.5 MODEL QUESTIONS

1. Describe the path of water from the time it enters the root until it escapes as water vapor from the leaf surface.
2. Write short notes on
   a) Cavitation and embolism
   b) Root pressure
   c) Cohesion theory of ascent of sap
   d) Anatomy of water conduction "

2.6 REFERENCE BOOKS

1. Introductory plant physiology GR. Noggle and G.J.Fritz.Prentice Hall of India -New Delhi. -
2. Plant Physiology-R.M. Devlin and F.H.Witham -CBS Publishers and distributors-New Delhi
3. Plant Physiology - F.B. Salisbury and C.W.Ross - CBS Publishers - New Delhi
4. Plant Physiology - L.Taiz: and E.Zeiger - Sinauer Associates, Inc., Publishers, Suderland, Massachusetts.
5. Introduction to Plant Physiology - W.G. Hopkins. John Wiley & Sons. Inc - New York.

Dr. C.V.S Bhaskar

LESSON -3

SOLUTE TRANSPORT BY ACTIVE AND PASSIVE MECHANISMS

OBJECTIVE:

In this lesson mineral salt absorption by diffusion, facilitated diffusion and active transport, membrane transport proteins such, as-S channels, carriers and pumps, symplast and apoplastic transport of ions from the roots to the shoots are discussed.

STRUCTURE OF THE LESSON:

3.1 INTRODUCTION

Except C, H, 0 all the remaining elements are present in the soil solution in dissociated condition so that plants adsorb them in the form of ions. Mineral ions are found either as soluble fractions of soil solution or as adsorbed ions on the surfaces of negatively charged inorganic and organic soil particles. Mineral cations such as ammonium (NH4 +), potassium (K+),magnesium (Mg2+), calcium (Ca2+), manganese (Mn2+)and others adsorb to the negative surface charges of soil particles.

These ions are not easily lost when the soil is leached by water, and they 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 capacity. For example, H+ replaces K+. A soil with higher cation exchange capacity generally has a larger reserve of mineral nutrients.

Mineral anions such as N03 -, Cl, SO^{-4}, and others occur as soluble fractions of soil solution in low concentrations and are potentially available for absorption by plant roots. Because of their negative charges, however, these ions will be subjected to repulsion with negatively charged soil particles so that they may be leached by water moving through the soil if not absorbed by the plant roots.

As we know, plant cells are separated from their environment by a plasma membrane. Plasma membrane is made up of proteins and non-polar lipids. It acts as a biological barrier for the free passage of charged solutes and ions in and out of the cell. The transport of charged nutrients across this biologically impermeable membrane takes place through the transport

proteins. Molecular and ionic movement between the soil and the plant and from cell to cell across the membrane is called transport or absorption. From time-to-time various theories have been proposed by different workers to explain the mechanism of ion absorption. These different theories are, however, categorized into three fundamental concepts such as diffusion, facilitated diffusion and active transport. These three processes now make up the basic language of transport across the membrane of all organisms.

These three basic models of transport are schematically shown in the Figure 5.1 and are described in detail in the following sections.

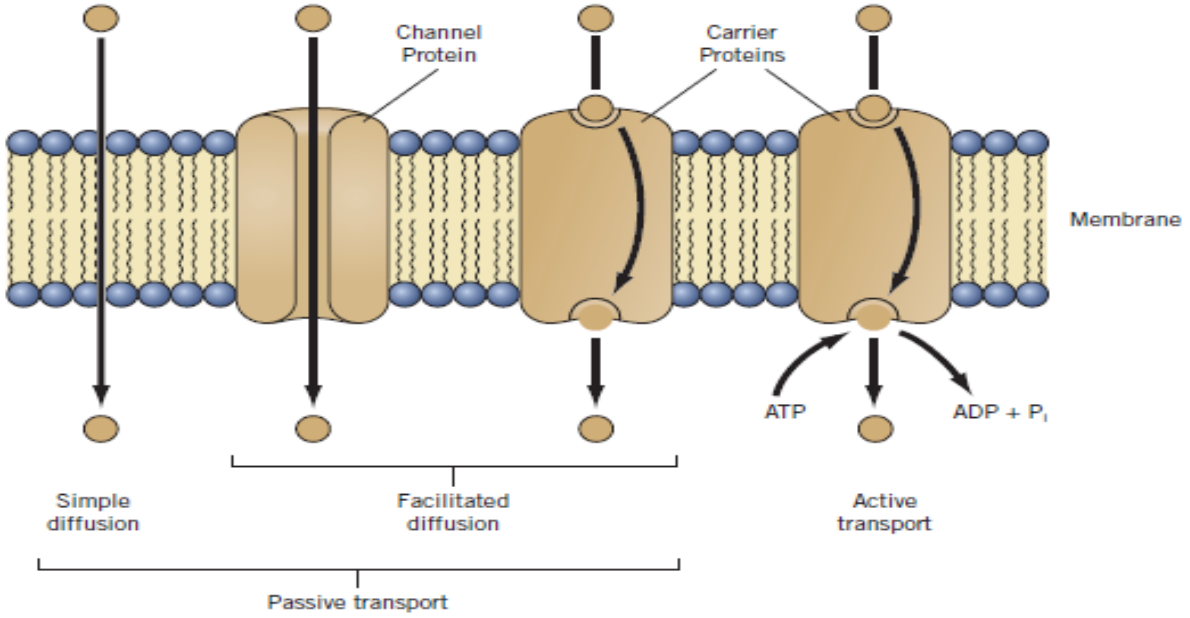


Figure 3.1 The transport of ions and solutes across membranes by simple diffusion, facilitated diffusion and active transport'

3.2 SIMPLE DIFFUSION

Diffusion is the process by which molecules intermingle because of their random thermal agitation. Such agitation gives rise to the random but progressive movement of substances from regions of high concentration (or high free energy) to regions of low concentration (or low free energy) down a concentration gradient. Fide discovered that the rate of diffusional movement of molecules is directly proportional to the concentration gradient. This law in symbols for a membrane bound cell
can be written as :

$$Js = - PA (C_s^{o} - C_s^{i})$$

Where Js is 'the flux density or rate of transport which specifies the amount of substance s crossing a unit membrane per unit time. P is the permeability coefficient that measures the velocity (in cm^{-1} S^{-1}) with which the substance **s** moves through the membrane. C_s^{o} - C_s^{i} is the concentration difference of substance s between outside and inside of the cell.· The negative *sign in* the equation .be faster when the concentration gradient becomes steeper or when the permeability coefficient 1S increased. Because the membrane is Lipid in character and those substances that have high permeability coefficient such as nonpolar substances moves rapidly across the biological membrane.

Nonpolar' substances such as 02' CO2 and NH3 enter the cell through simple diffusion process. Water is a highly polar molecule. Its permeability coefficient in lipid layer is very low. Although it is a polar molecule it diffuses rapidly and freely across the membranes from its high concentration to low concentration. Previously it was suggested that the free movement of water across the membranes takes place by simple diffusion. However, the recent molecular genetic studies indicated that the water transport occurs by membrane integrated proteins which form a water-selective channels across the membrane. These channel forming proteins are now called aquaporins. Aquaporins facilitate water movement across the membrane in response to the water potential gradient on either side of the membrane (Figure 5.2). Just how aquaporins achieve a high degree of selectivity for water remains unclear.

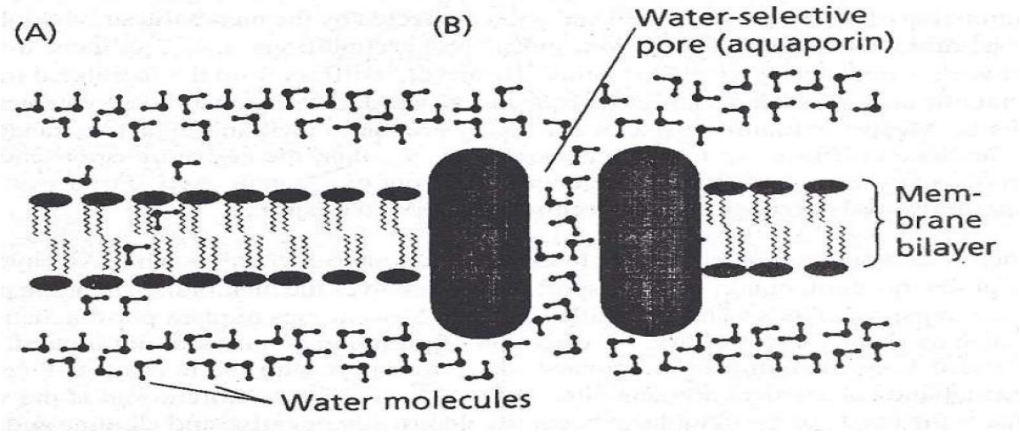


Figure 3.2 Water movement through a water selective pore formed by integral membrane protein aquaporins

3.3 FACILITATED DIFFUSION

The plasma membrane will not permit the free passage of most substances particularly charged solutes or ions. However, it was found that certain charged ions entered cells far more quickly than would be expected based on their diffusion through a lipid bilayer. The reason is that biological membranes contain transport proteins that facilitate the passage of ions and other polar molecules.

The rate of diffusion of certain solutes across selectively permeable membranes is therefore greatly increased by using transport proteins, sometimes called carriers, permeases and transporters. It is truly a diffusion process by which charged nutrients can cross the membrane with the help of membrane integrated carrier proteins. This carrier aided diffusion process is called facilitated diffusion. Each carrier is selective and will transport only closely related solutes. A concentration gradient spanning the membrane drives the movement of molecules. If the concentration gradient disappears, net inward movement ceases. Remember that this transport is reversible, if the solute concentration is greater inside the cell that is the solute will move outward.

The movement of molecules by either simple or facilitated diffusion across the plasma membrane is called passive transport. In passive transport molecules will always proceed spontaneously down a concentration or chemical gradient until equilibrium is reached without a direct input of metabolic energy. These processes do not cause an accumulation of solu against its concentration or electrochemical gradient.'

3.4 ACTIVE TRANSPORT

The movement of solute molecules against a concentration or chemical gradient is termed active transport. It is not a spontaneous process and requires input of metabolic energy. This transport will lead to an accumulation of solute inside the cell and will be affected by the metabolic activity inhibitors such as low temperature, inhibitors of respiration, and anaerobic conditions. Like facilitated diffusion, active transport is also mediated by carrier proteins. However, it differs from the facilitated transport in its use of metabolic energy and in its ability to concentrate substances against their concentration or chemical gradients. Metabolic inhibitors that block energy production will inhibit active transport but will not affect facilitated diffusion at least for a short time. Further, active transport causes the movement of molecules in only one direction either into or out of the cell. Active transport can be bisected into primary and secondary active transport.

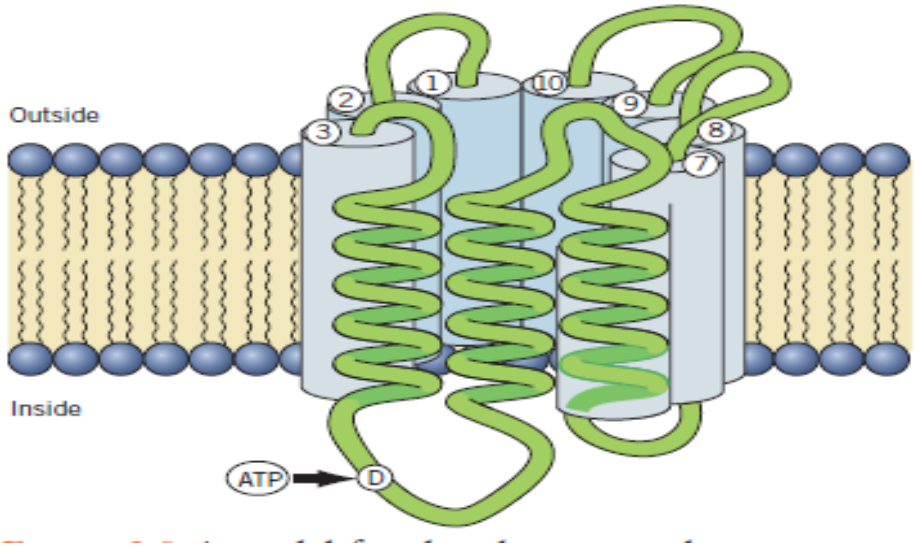


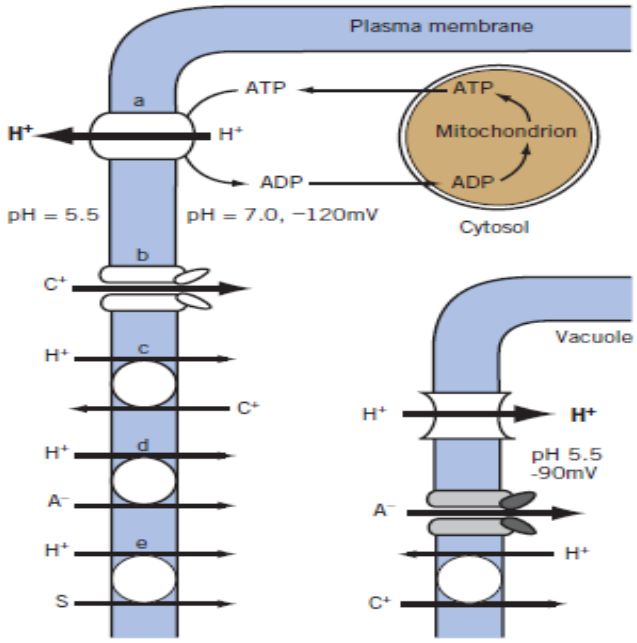
FIGURE 3.3 A model for the plasma membrane H+-ATPase. The enzyme is a single chain with 10 hydrophobic, membrane-spanning domains. Only three are shown here as helical coils, while the remaining seven are schematically represented as cylinders. Linking adjacent membrane-spanning domains are hydrophilic loops that project into the cytosol (inside) and the cell wall apoplast (outside). The ATP-binding site is an asparticnacid residue (*D*) located on the hydrophilic loop between the fourth and fifth membrane-spanning domains. The H+-binding site is located in the hydrophobic domain.Hydrolysis of ATP at the binding site is thought to change the conformation of the enzyme, thereby exposing the H+-binding site to the outside of the membrane where the H+ is released.

Active transport requires a direct input of metabolic energy, it is sensitive to oxygen and respiratory poisons. Thus reduced uptake of a particular ion in the absence of oxygen or in the presence of respiratory inhibitors such as cyanide or 2,4-dinitrophenol would be evidence in support of active transport. Even this evidence is not always compelling,however, since effects of inhibitors may indirectly influence nutrient uptake. For example, even transport by diffusion ultimately requires an expenditure of metabolic energy, if only to establish and maintain the organization of membranes and other properties of the cell that make transport possible. It is not always a simple matter to distinguish between direct and indirect involvement of energy. The criteria for active transport usually require that the solute distribution not be in electrochemical equilibrium and that there be a quantitative relationship between energy expended and the amount of solute transported.

Finally, it should be noted that when ion concentrations are known, the Nernst equation can also be used to estimate the transmembrane potential, or Nernst potential, contributed by that ion. At steady state, however, calculation of the membrane potential is complicated by the fact that many different ion species, each with a different permeability, are simultaneously crossing the membrane in both directions. The individual contributions of all ion gradients must consequently be taken into account and summed in order to arrive at the overall potential for the cell. In practice, however, K+, Na+, and Cl− are the dominant ions. These ions have the highest permeabilities and concentrations in plant cells and a reasonable estimation of transmembrane potential can be based on these three ions alone.

ACTIVE TRANSPORT IS DRIVEN BY ATPASE-PROTON PUMPS

Energy to drive active transport comes chiefly from the hydrolysis of ATP. The energy-ransducing membranes of chloroplasts and mitochondria contain large, multiprotein ATPase complexes . The chloroplast and mitochondrial ATPases, known as F-type ATPases, utilize the energy associated with an electrochemical proton gradient across a membrane to drive ATP synthesis. ATPase complexes are also found in the plasma and vacuolar membranes of cells and possibly other membranes as well. These ATPases are called ATPase-proton pumps (or, H+-ATPase). Known as P-type ATPases, the plasma membrane ATPase-proton pumps are structurally distinct (Figure 3.9) and operate in reverse of the F-type. Rather than synthesizing ATP, the plasma membrane ATPases hydrolyze ATP and use the negative free energy to ‘‘pump’’ protons from one side of the membrane to the other against an electrochemical gradient. An ATPase-proton pump thus serves as a proton-translocating carrier protein and the free energy of ATP hydrolysis is conserved in the form of a proton gradient across the membrane.This proton gradient (pH), together with the normal membrane potential, contributes to a proton motive force (pmf) that tends to move protons back across the membrane



Schematic diagram relating the activity of a membrane ATPase-proton pump to solute exchange. The proton pump (*a*) uses the energy of ATP to establish both a proton gradient and a potential difference (negative inside) across the membrane. The energy of the proton gradient may activate an ion channel (*b*), or drive the removal of ions from the cell by an

antiport carrier (*c*), or drive the uptake of ions or uncharged solute by a symport carrier (*d*, *e*). Similar pumps and carriers operate across the vacuolar membrane. C+, cation; A−,anion; S, uncharged solute.

It is generally conceded that the proton motive force established by pumping protons across membranes is the primary source of energy for a variety of plant activities.We will revisit this equation and the concept of a proton motive force in our discussion of ATP synthesis in the chloroplast and the mitochondrion Included are activities such as active transport of solutes (cations, anions, amino acids, and sugars),regulation of cytoplasmic pH, stomatal opening and closure, sucrose transport during phloem loading, and hormone-mediated cell elongation. A schematic model relating ATPase-proton pumps to solute exchange across membranes The ATP required to drive the pump is ultimately derived from oxidative phosphorylation in the mitochondria. The proton-translocating protein is shown extending across the plasma membrane, with its ATP binding site on the cytosolic side. Hydrolysis of the ATP results in the translocation of one or more protons from the cytosol to the surrounding apoplastic cell wall space.

There are several particularly interesting consequences of the ATPase-proton pump. First, a single ion species is translocated in one direction. This form of transport is consequently known as a **uniport** system. Second, because the ion transported carries a charge, an electrochemical gradient is established across the membrane. In other words, the ATPase-proton pump is **electrogenic**—it contributes directly to the negative potential difference across the plasma membrane. In fact, the electrogenic proton pump is a major factor in the membrane potential of most plant cells. From equation 3.5, a tenfold difference in proton concentration (one pH unit) at 25◦C contributes 59mV to the potential. Since the proton gradient across the plasma membrane is normally on the order of 1.5 to 2 pH units, it can account for approximately 90 to 120mV of the total membrane potential. Third, since the ions translocated are protons, the ATPase-proton pump establishes a proton gradient as well as an electrical gradient across the membrane. Energy stored in the resulting electrochemical proton gradient (or the *proton motive force*) can then be coupled to cellular work in accordance with Mitchell's *chemiosmotic hypothesis* . Indeed, this is an excellent demonstration of how chemiosmotic coupling is not restricted to ATP synthesis in chloroplasts and mitochondria but can be used to perform other kinds of work elsewhere in the cell.

Several ways of coupling the electrochemical proton gradient to solute movement across the membrane are illustrated in Figure 3.10. In the first case, the electrogenic pump contributes to the charge-dependent uptake of cations through ion-specific channels .(b)). Second, the return of protons to the cytosol can be coupled with the transport of other solute molecules at the same time, or **cotransport**. Transport of both ions is mediated by the same carrier protein and the movement of the second solute is obligatorily coupled to the inward flux of protons down their electrochemical gradient.If the second ion moves in opposite direction from the proton, the method of cotransport is referred to more specifically as **antiport**. for example, proton flux into the cell is shown coupled to the efflux of other cations out of the cell. Here the energy of the proton electrochemical gradient is used to maintain low internal concentrations of specific cations. Any cations that do chance to ''leak'' into the cell, no doubt passively through ion channels, are thus pumped out against their electrochemical gradient. If the two solutes move in the same direction at the same time, the method of cotransport is referred to as **symport.**In the first example, proton flux into the cell is coupled with the uptake of anions

(A−) against their electrochemical gradient. In the second example of symport, the proton gradient can be used to power the uptake of uncharged solutes (S), such as sugars. All three examples of cotransport are forms of active transport mediated by specific carrier proteins.

Primary active transport is coupled directly to a metabolic source of energy, such as ATP hydrolysis, or some other high-energy compound. This transport system involves the membrane-spanning proteins to carry out active transport of ions. They are called pumps. Most pumps of plant plasma membranes transport ions such as H+ or Ca2+. Further, the direction of pumping is outward, not inward.

When protons are extruded from the cytosol by primary active transport with the help of ATP energy, a membrane potential and pH gradient are generated both at the plasma membrane and at the vacuole membrane. That is the inside of the cytoplasm becomes electrically negative and alkaline and the outside of the membrane becomes electrically positive and acidic. This gradient of electrochemical potential for H+ across the membrane is called proton motive force (PMF or Ap), It represents stored free energy in the form of the H+gradient. The proton motive force generated by primary active transport is used to drive the transport of many other organic and essential mineral nutrients against their concentrations by another transport mechanism called the secondary active transport. It is a carrier mediated co-transport system. In this mechanism the solutes are actively transported across a membrane against their electrochemical potential gradients by coupling of the uphill transport of one solute to the downhill transport of another.

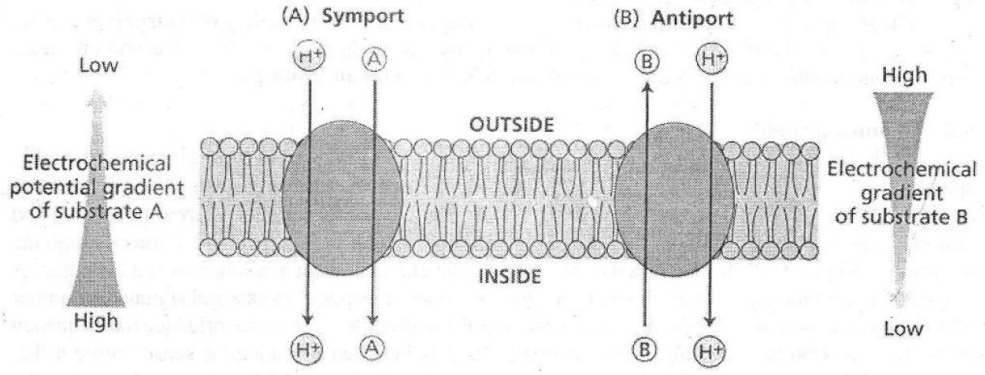


Figure 3.3 Secondary active transport types. (A) Symport system. The energy dissipated by a proton moving back into the cell is coupled to the uptake of one substrate molecule into the cell. (B) Antiport system. The energy dissipated by a proton moving back into the cell is called the active transport of the solute out of the cell. In both cases, the substrate under consideration is moving against its electrochemical potential gradient.

The secondary active transport is of two types: the symport system and the antiport system A symport simultaneously transport two different substances across the membrane in the same direction. In plants and fungi, the sugars and amino acids are taken up by symport with protons. For example, when glucose is supplied to a plant cell bathed in a simple solution of mineral salts, a reduction in the membrane potential, an increase in external pH, and uptake of glucose occur simultaneously. The decrease in membrane potential is due to the positive charges (H+) that move into the cell along with glucose. However, this membrane depolarization is transitory, because the reduced membrane voltage allows the primary active transport H+ pump to work faster and thereby restore the membrane voltage and pH gradient

in the presence of continuing glucose uptake. Similarly, transport of Cl, NO_3-, $H_2PO_4^-$, K^+, sucrose, amino acids, and other substances enter the cell via specific secondary active proton symport systems.

An antiport simultaneously transports two different molecules across the membrane in opposite directions. For example, sodium transport across the membranes takes place by secondary active antiporter system in which sodium is transported out of the cell in response to the inward movement of protons.

In both types of secondary transport, the ion or solute being transported along with the protons is moving against its gradient-of electrochemical potential, so that its transport is active. However, the energy driving this transport. is provided by the proton motive force rather than by ATP hydrolysis. Typically, a cell depends on ATP driven primary active transport system in order to set up the proton motive force. Many other ions and organic substrates can then be transported by a secondary active transport system with the help of proton motive force i.e by simultaneously carrying one or two H^+down their energy gradient. Thus H^+ions circulate across the membrane, outward through the primary active transport, and back into the cell through the secondary transport system.

IONS ARE ACTIVELY SECRETED INTO THE XYLEM APOPLAST

With the exception of the very tip of the root where the young xylem vessel elements are still maturing, functional xylem is part of the apoplast. The interconnected vessel elements are devoid of cytoplasm and consist only of nonliving tubes filled with an aqueous solution. Release of ions into the xylem thus requires a transfer from the symplast into the apoplast. At one time, it was thought that this transfer was simply a passive leakage,but it is now clear that ions are actively secreted from xylem parenchyma cells.

Although there is some conflicting evidence, ion concentration in the apoplast of the stele is generally much higher than in the surrounding cortex. This suggests that ions are being accumulated in the xylem against a concentration gradient, presumably by an energy-dependent, carrier-mediated process.It is also interesting to speculate, in this regard, that the Casparian band also functions to prevent loss of ions from the stele by blocking their diffusion down a concentration gradient.

In addition to working uphill against a concentration gradient, delivery of ions into the xylem vessels is sensitive to metabolic inhibitors such as carbonyl-cyanide-m-chlorophenylhydrazone (CCCP), which uncouples ATP formation. It is interesting that ion transport into the xylem is also sensitive to cycloheximide, an inhibitor of protein synthesis, but uptake into the root, at least initially, is not affected.

Two plant hormones (abscisic acid and cytokinin) have a similar effect. Whether inhibitors of protein synthesis and hormones are affecting symplastic transport through the endodermis or unloading of ions from the endodermis into the xylem is not certain, but these results at least raise the possibility that ion release into the vessels is a different kind of process than ion uptake by the roots.

EMERGING SECONDARY ROOTS MAY CONTRIBUTE TO THE UPTAKE OF SOME SOLUTES

The possibility remains that a limited portion of ion uptake may be accomplished entirely through the apoplast, at least in some roots. More basal endodermal cells—the distance from the tip is variable, but measured in centimeters—are characterized by additional suberin deposits that cover the entire radial and inner tangential wall surfaces. This would seem to present an additional barrier to apoplastic flow.

However, in some plants, a small number of endodermal cells, called passage cells, remain unsuberized. Passage cells might represent a major point of entry for solutes into the stele.Apoplastic continuity between the cortex and stele may also be established at the point of lateral root formation. One series of experiments, for example, followed the path of fluorescent dyes into the vascular tissues and shoots of corn (Zea mays) and broad bean (Vicia faba) seedlings. These dyes were chosen because they cannot be taken up by cells and thus are normally confined to the apoplast, The point of dye entry was traced to recently emerged secondary roots. These branch roots arise in the pericycle, a layer of cells immediately inside the endodermis. The emergence of the root primordia through the endodermis disrupts the continuity of the Casparian band and establishes, at least temporarily, the apoplastic continuity required to allow diffusion of the dye into the vascular tissue. Continuity of the apoplast through passage cells and secondary roots has been cited to explain increased calcium uptake in certain regions of corn roots. It may also help to account for the fact that a plant appears to contain virtually every element that is found in its environment, even those not known to be essential or not accumulated by plant cells.

The uptake of ions is not uniform along the length of the root. As shown in Table 3.1, uptake of calcium is highest in the apical 3 cm of the root while potassium is taken up in roughly equivalent amounts along the first 15 cm. Moreover, most of what is taken up in the tip (almost two-thirds of the calcium and three-fourths of the potassium) remains in the root. The proportion of ions translocated to the shoot increases with increasing distance from the tip. It is also interesting that when calcium is taken up further along the root (12 to 15 cm from the tip), it is translocated to the shoot but not to the tip. Clearly, although substantial progress has been made in several laboratories, the transport of ions through roots and into the xylem remains a complex and challenging field of study.

TABLE 3.1 Uptake and translocation of potassium and calcium as a function of position along a corn root.

Zone of application[1]	Ion	Total Uptake[2]	Percent Retained	Percent Translocated to:	
				Root Tip	Shoot
0–3	K^+	15.3	75	—	25
	Ca^{2+}	6.3	63	—	37
6–9	K^+	22.7	17	19	64
	Ca^{2+}	3.8	42	—	58
12–15	K^+	19.5	10	10	80
	Ca^{2+}	2.8	14	—	86

[1]Distance from root tip, cm.
[2]Uptake expressed as microequivalents per 24 hours.
Based on data of H. Marschner and C. Richter, 1973, Z. Pflenzenernaehr, Bodenkd, 135:1–15.

3.5 SUMMARY

Solute transport across cell membranes occurs through two primary mechanisms: passive transport, which moves solutes along their concentration gradient without energy, and active transport, which moves solutes against their concentration gradient using cellular energy (ATP). These processes are vital for maintaining cellular balance (homeostasis).

Passive transport is the movement of ions and molecules across the cell membrane without requiring cellular energy (ATP). It relies on the natural kinetic energy of molecules and the presence of a concentration gradient, moving substances from a region of higher concentration to a region of lower concentration until equilibrium is reached.

Mechanisms of Passive Transport : Simple Diffusion: Small, nonpolar, and lipid-soluble molecules like oxygen pass directly through the lipid bilayer of the cell membrane.

Facilitated Diffusion: Larger or polar molecules (e.g., glucose, amino acids, ions) move across the membrane with the assistance of specific transmembrane channel proteins or carrier proteins. This still follows the concentration gradient and requires no energy input from the cell. Osmosis: This is the specific diffusion of water molecules across a selectively permeable membrane from an area of higher water concentration (lower solute concentration) to an area of lower water concentration (higher solute concentration).

Active Transport: Active transport is the process by which cells move molecules or ions against their concentration gradient, from an area of lower concentration to an area of higher concentration. This process requires an input of cellular energy, typically in the form of ATP (adenosine triphosphate), and involves specialized carrier proteins known as pumps.

Mechanisms of Active Transport: Primary Active Transport: Energy from the direct hydrolysis of ATP is used to pump molecules against their gradient. A classic example is the sodium-potassium pump (Na-ATPase) found in nerve and muscle cells, which moves three sodium ions out of the cell and two potassium ions into the cell.

Secondary Active Transport (Coupled Transport): This mechanism uses the energy stored in an electrochemical gradient (created by primary active transport) to move another substance against its own gradient. Symport: Both substances move in the same direction across the membrane (e.g., glucose and sodium uptake in the intestines). Antiport: The substances move in opposite directions (e.g., the sodium-calcium exchanger).

Vesicular Transport (Endocytosis and Exocytosis): This involves the transport of large quantities of material or large particles (like proteins or entire cells) by engulfing them in membrane-bound sacs called vesicles. This is an energy-intensive process.

3.6 MODEL QUESTIONS

1. Differentiate between active and passive transport based on criteria such as energy requirement (ATP),
2. Compare and contrast simple diffusion, facilitated diffusion, and active transport.
3. Define osmosis and Explain the term 'facilitated diffusion in the context of passive transport.

4. What drives molecules in passive transport?".
5. Which is not a type of passive transport? .
6. Mention two examples of active and passive transport in biological systems
7. What are the three types of membrane transport proteins involved in passive and active transport**?**

3.7 SUGGESTED READINGS

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Dr CVS Bhaskar

LESSON -4

STRUCTURE AND PROPERTIES OF MEMBRANE TRANSPORT PROTEINS

OBJECTIVE :

In this lesson membrane transport proteins such as -S channels, carriers and pumps, symplast and apoplastic transport of ions from the roots to the shoots are discussed**.**

STRUCTURE OF THE LESSON:

4.1 MEMBRANE TRANSPORT PROTEINS

The transport of ions and solutes across the biological barrier by a variety of transport processes as discussed above involves several classes of membrane-spanning proteins. These transport proteins can be grouped into three main categories: Channels, Carriers and Pumps.

4.2 CHANNEL PROTEINS

Channels are transmembrane ion or solute transport proteins. Most of these channels are highly specific for one or a limited number of ion species, which can diffuse through an open channel at rates as high as 108 S-I. Channel proteins may exist in two different conformations referred to as open and closed. In the open conformation, the core of the protein forms a pore for diffusion of ions through the membrane (Figure 4.1). In the closed confirmation, the channel is not available for ion diffusion. A channel may contain a gate that can open and close the pore in response to external signals. A few signals including voltage, light, hormones and ions themselves, are known to influence the frequency or duration of channel opening. The channel protein is believed to contain a sensor protein that· responds to the appropriate stimulus by changing the conformation of the protein and opening the gate.

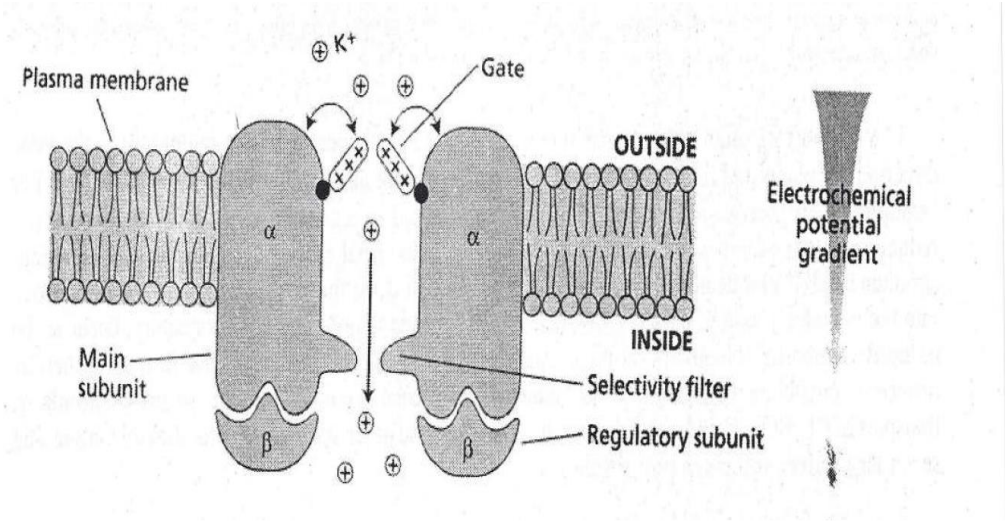


Figure 4.1 A model of a voltage gated K^+channel in a plant plasma membrane. The gate opens or. closes 'the pore in response to the membrane potential. Selectivity filters and regulatory subunit may also be present.

Transport through a channel is always passive. Its specificity of ion transport depends on pore size and electric charge rather than on selective binding. The region of the channel that determines its specificity is called the selectivity filter.

In guard cell plasma membranes, two types of gated anion channels have been distinguished based on how long the gates remain open in response to a prolonged stimulus. They are designated as R-type channels (rapidly activated) and S-type channels (slowly activated). R-type channels. open and close very rapidly in response to a voltage stimulus while S-type channels remain open for the duration of the stimulus. Rapid and slow type channels have also been identified in vacuolar membranes, and they are referred to as fast vacuolar (FV) or slow vacuolar (SV) channels.

4.3 CARRIER PROTEINS

In the 1930s, students of membrane transport recognized that certain ions entered cells far more rapidly than would be expected on the basis of their diffusion through a lipid bilayer. We now know that thismis because natural membranes contain a large number of proteins, many of which function as **transport proteins**.Some of these transport proteins *facilitate* the diffusion of solutes, especially charged solutes or ions,into the cell by effectively overcoming the solubility problem. The term **facilitated diffusion** was coined to describe this rapid, assisted diffusion of solutes across the membrane. In facilitated diffusion, as in simple diffusion, the direction of transport is still determined by the concentration gradient (for uncharged solute) or electrochemical gradient (for charged solutes and ions).

Facilitated diffusion is also bidirectional and, like simple diffusion, net movement ceases when the rate of movement across the membrane is the same in both directions. Two major classes of transport proteins are known. **Carrier proteins** (also known as **carriers, transporters**, or simply, **porters**) bind the particular solute to be transported, much along the lines of an enzyme–substrate interaction. Binding of the solute normally induces a conformational change in the carrierprotein, which delivers the solute to the other side of the membrane. Release of the solute at the other surface of the membrane completes the transport and the protein then reverts to its original conformation, ready to pick up another solute.

Channel proteins are commonly visualized as a charged-lined, water-filled channel that extends across the membrane. Channels are normally identified by the ion species that is able to permeate the channel, which is in turn dependent on the size of the hydrated ion and its charge. Diffusion through a channel is dependent on the *hydrated size* of the ion because the associated water molecules must diffuse along with the ion. The number of ion channels discovered in the membranes of plant cells is increasing. Currently there is solid evidence for K+, Cl−, and Ca2+ channels, while additional channels for other inorganic and organic ions are strongly suggested.

Channel proteins are frequently **gated**, which means they may be open or closed (Box 3.1). Solutes of an appropriate size and charge may diffuse through only when the channel ‘‘gate’’ is open. Two types of gates are known. An electrically gated channel opens in response to membrane potentials of a particular magnitude.

Other channels may open only in the presence of the ion that is to be transported and may be modulated by light, hormones, or other stimuli. The precise mechanism of gated channels is not known, although it is presumed to involve a change in the three-dimensional shape, or conformation, of the protein. The importance of carriers lies in the selectivity they impart with respect to which solutes are permitted to enter or exit the cell. Channels, on the other hand, appear to be involved wherever large quantities of solute,particularly charged solutes or ions, must cross the membrane rapidly. Whereas a carrier may transport between 104 and 105 solute molecules per second, a channel may pass on the order of 108 ions per second. It should also be stressed that large numbers of channels are not required to satisfy the needs of most cells. The rate of efflux through guard cell K+ channels during stomatal closure, for example, has been estimated at 107 K+ ions sec−1—a rate that conceivably could be accommodated by a single channel. Many carrier and channel proteins are inducible, which means that they are synthesized by the cell only when there is solute available to be taken up.

Carrier proteins are also known as transporters or simply porters. In transport mediated by a carrier, the solute or ion being transported is initially bound to a specific site on the carrier protein like an enzyme-substrate binding. Binding of the solute causes a conformational change in the carrier protein, which exposes the solute to the solution on the other side of the membrane. Release of the solute at the other surface of the membrane completes the transport and the protein then reverts to its 'original conformation. ready to pick up another solute. Typically, carriers may transport 100 to 1000 'ions or molecules per second, which is about 106 times slower than transport through a channel.

Carrier-mediated transport can be either passive or active. Facilitated diffusion, a passive process that takes place through these carriers," transport the substances down their concentration gradients without an additional input of metabolic energy. Carrier mediated secondary active transport involves two types of transmembrane carriers called **symporters and antiporters.** These carriers contain two sites on the outside of the membrane to bind a proton and a solute. Proton binding at the first site causes the second site to be exposed, this site binds the ion or solute that is being actively transported. With both molecules bound, the transporter undergoes a conformational change that exposes the binding" sites to the opposite side of the membrane. The cycle is completed by diffusion of the proton and substrate molecules away from their binding sites, causing the transporter to regain its original, or "relaxed" confirmation (Figure 4.2).

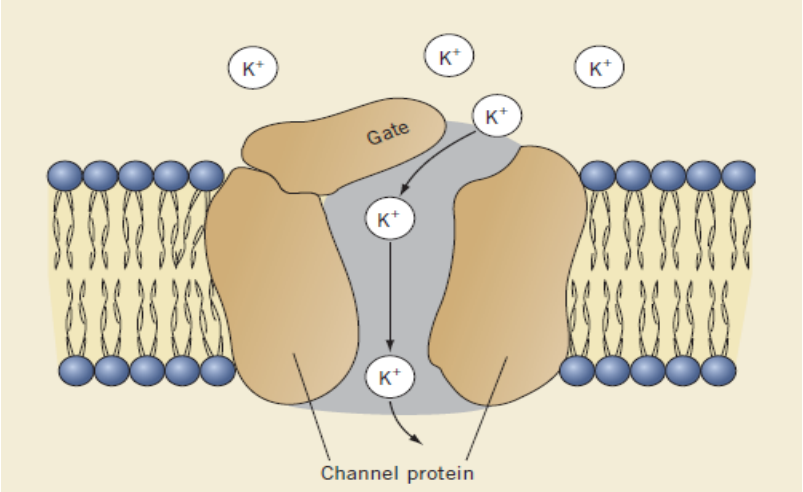


FIGURE 4.2 A gated membrane channel. Gated channels may be open, in which case ions are permitted to pass through the channel, or closed to ion flow. Opening may be stimulated by hanges in membrane potential, the presence of hormones, or the ion itself.

4.4 PUMPS

The membrane proteins that carry out primary active transport are called pumps. The pumps can use the energy of ATP hydrolysis to establish a proton gradient across the membrane. Hence, they are also known as **ATPase-proton pumps.** These pumps are large multiprotein complexes found in the plasma membranes and tonoplasts. ATPase-proton pumps may be either **electrogenic** or **electro neutral.** Electrogenic pumps cause the net movement of charge across the membrane, while electroneutral pumps, as the name implies, involve no net movement of charge. For example, Na^+/ K+ - ATPase of animal cells pumps three Na" ions out for every two K+ ions in, resulting in net outward movement of one positive charge. The Na'? K+ - ATPase is therefore an electrogenic ion pump. In contrast, the H+/ K+ - ATPase pumps one H+ out of the cell for every one K+ in, so there is no net movement of charge across the membrane. Therefore, the H+/K+- ATPase is an electroneutral pump.

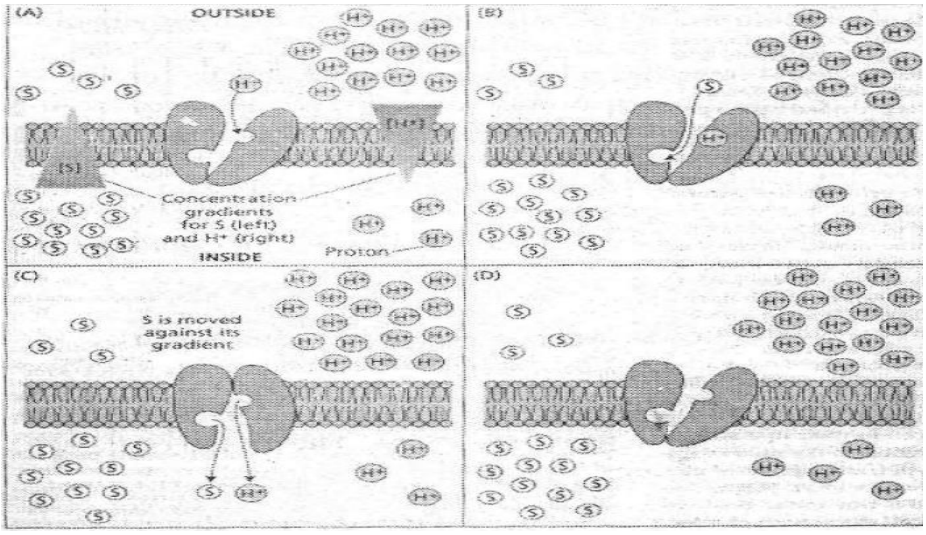


Figure- 4.2 A model showing the mechanism of secondary active transport. (A) Initial conformation of the carrier with binding sites on the 'outside of the membrane. (B) H^+binding exposes the substrate binding site of the carrier. (C) The binding of H^+ and S^- cause the

Carrier to be present in another conformation that exposes both the sites to the inside of the cell. (D) Release of a proton and substrate into the cytosol restores the original conformation of the carrier. "

H+- ATPase is the electrogenic proton pump of plant plasma membranes. This pump creates the proton gradient across the membrane by translocating protons from the cytosol to the surrounding apoplastic cell wall space with the use of ATP energy. This proton gradient, together with the normal membrane potential, establishes a proton motive force that tends to move protons back across the membrane. This proton motive force is the primary source of energy to drive the secondary active transport, necessary for the active transport of many other substances.

Figure 4.3 shows how a plasma membrane H+- ATPase might work. It contains ATP and proton binding sites. When it is at rest, both these sites are present on the cytosolic side with an occluded pore in the membrane (A). When ATP and proton are bound at these sites, a phosphate group from ATP is transferred to specific amino acid residue aspartic acid on the protein (B). This phosphorylation then causes the protein to undergo a conformational change, opening the transport pathway to the outside and simultaneously closing it on the cytosolic side (C). The proton then leaves its binding site, and the protein 'comes to its original conformation with the removal of phosphate group (D).

ATPases that are Phosphorylated as part of the catalytic cycle are known as P-type ATPases.

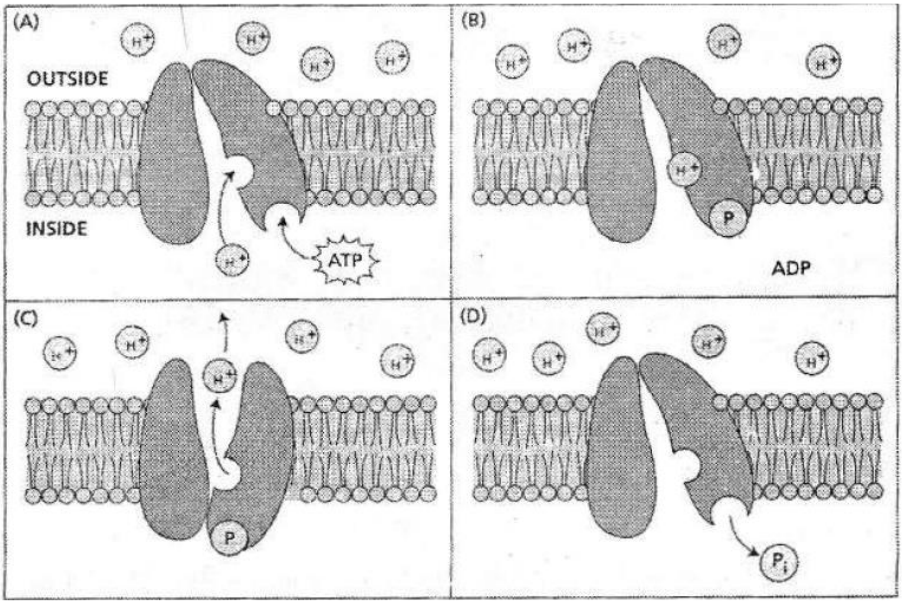
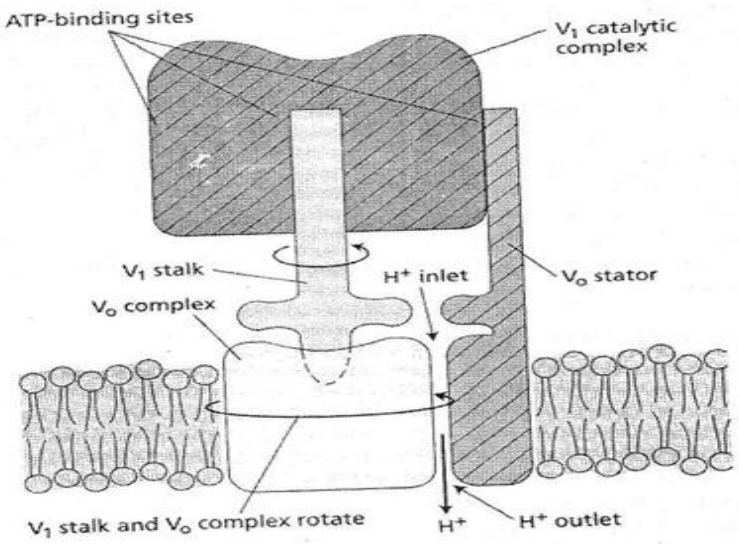


Figure 4.3 A model showing the transport at H+ against electrogenic gradient by an electromagnetic H+ pump.

According to molecular studies, the plasma membrane H+-ATPase consists of a single polypeptide chain with a molecular mass of 100 kDa. Different tissues of the plant have various H+-ATPase isoforms to regulate transport in different ways for each tissue. Like other enzymes, the plasma membrane H+-ATPase is regulated by the concentration, of substrate (ATP), pH and temperature. Further, H+- ATPase causes the translocation of a single ion (H+)in one direction. This kind of transport is called **uniport system** and H+-ATPase as **uniporter protein.**

Tonoplasts containing H+-ATPase is known as **vacuolar H+-ATPase or V-ATPase.** This electrogenic pump translocate protons from the cytoplasm into the lumen of vacuole. V-ATPase is a large enzyme complex made up of at least ten different subunits with a molecular mass of about 750 kDa. It differs both structurally and functionally from the plasma membrane H+-ATPase because it does not involve the formation of a phosphorylated intermediate. Like F-ATPases of chloroplasts and mitochondria, the catalytic subunits of V-ATPases are organized into a peripheral catalytic complex, V_I' and an integral membrane channel complex, V_o' Figure 4.4 shows how ATPases might work. Hydrolysis of ATP by the V_I catalytic complex drives the rotation of the V_I stalk. The rotation of the V_I stalk, in turn, drives the rotation of the Vo complex. When the Vo complex turns, protons are transported from one side of the membrane to the other.



.Figure 4.4 Vacuolar H+-ATPase and its organization in the tonoplast.

tissue (or be carried passively by water flow) exclusively through the cell wall without crossing any membranes. The apoplast is the continuous system of cell walls and intercellular air spaces in plant tissues. Ions may also move via the symplast pathway, which consists of the entire network of cell cytoplasm interconnected by cytoplasmic bridges called **plasmodesmata.** These two pathways enable the ions to reach the endodermis, a boundary layer between the stele and the cortex. At the endodermis, water and ion movement into the stele through the apoplast pathway may be obstructed by the Casparian strip. The Casparian strip is a band of radial cell walls in the endodermis that is impregnated with the waxlike, hydrophobic substance suberin. Suberin acts as a barrier to water and solute movement. Hence the only possible route for ions to pass through the endodermis is to enter the symplast by some carrier or channel mediated transport at the cell membrane. In all cases, ions must enter the symplast before they can enter the stele because of the presence of Casparian strip. Symplastic connections facilitate ions passive movement from cell to cell until they arrive at a xylem parenchyma cell in the stele. At this point the ions are unloaded into the xylem vessels (Figure 4.5).

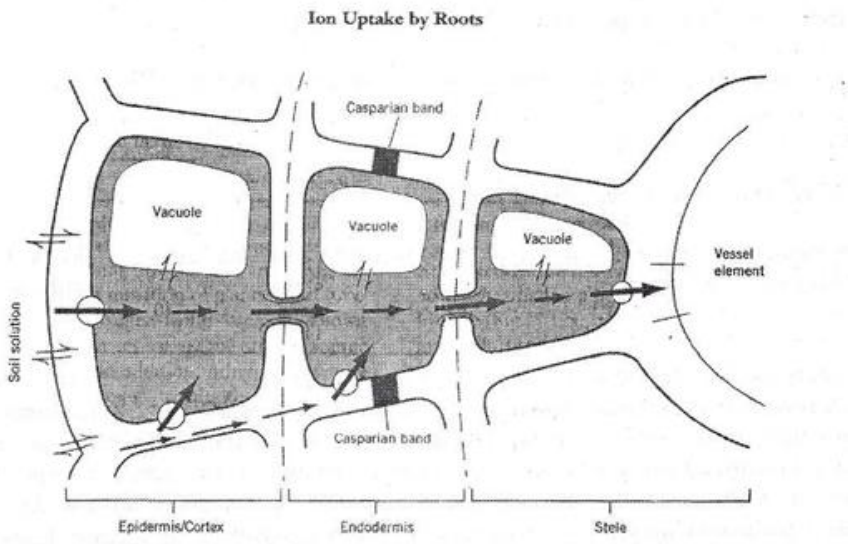


Figure 4.5 The radial path of ion movement through a root. Arrows indicate the alternate paths

that may be taken by nutrient ions as they move from the soil solution into the vascular elements in the stele. Arrows with circles indicate active transport of ions across plasma membranes

Once ions have been taken up into the symplast of the root at the epidermis or cortex, they must be loaded into the tracheid or vessel elements of the stele to be translocated to the shoot. Since the xylem tracheary elements are devoid of cytoplasm and consist only of non-living, water filled tubes, the ions must exit the symplast by crossing a plasma membrane a second time. Release of ions into the xylem thus requires a transfer from the symplast into the apoplast. The process whereby ions exist the symplast and enter the conducting cells of the xylem is called **xylem loading.** At one time, it was thought that this transfer was simply a passive leakage, but the evidence now suggests that ions are actively secreted from the xylem parenchyma. The plasma membranes of xylem parenchyma cells contain proton pumps, water channels, and a variety of ion channels specialized for influx or efflux. The flux of ions from the xylem parenchyma cells into the xylem tracheary elements, rather than being due to simple leakage, is under tight metabolic control through regulation of the plasma membrane H+ - ATPase and ion efflux channels.

4.5 ACTIVE AND PASSIVE ABSORPTION

The concentrations of some ions inside the cell may reach levels much higher than in the surrounding medium. This phenomenon is expressed quantitatively by the accumulation ratio which can be defined as the ratio of the concentration inside the cell (C') to the concentration outside the cell (C^O). For example, the internal concentration of K^+ in maize roots is more than 1000 times greater than it is in the surrounding nutrient solution. In the past, an accumulation ratio was greater than one has been considered compelling evidence in favour of active transport. Conversely, an accumulation ratio of less than one implies that the solute has been actively excluded or extruded from the cell. This is true for uncharged solutes like sugars. But for charged solutes or ions, the accumulation ratio is not always a valid indication to say whether the ion is accumulated either by passive or active channels are transport proteins that span the membrane, forming pores through which solutes diffuse down their

gradient of electrochemical potentials. Carriers bind a solute on one side of the membrane and release it on the other side.

In plants, a family of H^+-pumping ATPases provide the primary driving force for transport across the plasma membrane. Further, V-ATPases and H^+-Pyrophosphatase serve this function at the tonoplast. In addition, vacuolar membranes also contain ATP-binding cassette transporters that use the energy of ATP directly to transport large organic molecules into the vacuole. The gradient of electrochemical potential generated by H+pumping is used to drive the transport of other substances in a process called secondary transport. Secondary active transport is mediated by symporters and antiporters.

The relationship between the voltage difference across the membrane and the distribution of ion at equilibrium is described by the Nernst equation. Deviations from the concentrations prediated by the Nernst equation are considered evidence that either active uptake or expulsion of the ions is involved.

Solutes move between cells either through the apoplast or from cytoplasm to cytoplasm via the symplast. Cytoplasm of neighboring cells are connected by plasmodesmata, which facilitates symplast transport. When an ion enters the root, it may be taken up into the cytoplasm of an epidermal cell, or it may diffuse through the apoplast into the root cortex and enter the symplast through a cortical cell. From the symplast, the ion is loaded into the xylem and transported to the shoot.

4.6 ION TRANSPORT FROM ROOTS TO SHOOTS

So far, we have seen the ion transport at cellular level. In this section we will discuss the pathways by which ions can move from root to shoot via the xylem. Mineral nutrients absorbed by the root are carried to the shoot by the transpiration stream moving through the xylem. When an ion enters the root, it may be transported across the root into the xylem by apoplast pathway or symplast pathway. In the apoplast pathway, ions can diffuse across.

As we know, plant cells enlarge primarily by the uptake of water into the large central vacuoles so that the osmotic pressure of the vacuole must be maintained sufficiently high for water to enter from the cytoplasm. The electrogenic proton pumping V-ATPases of tonoplasts do this job. They can generate a proton motive force across the tonoplast by pumping protons' from 'the cytoplasm into the vacuole. The accumulation of H^+ inside the vacuole due to V-ATPase activity accounts for the fact that the pH of the vacuolar sap becomes low i.e about 5.5 compared to the cytoplasmic pH of 7.0 to 7.5. The electrical component of the resulting protonmotive force drives the uptake of anions such as CI^- and malate^{2+}" into the vacuole and the pH gradient is used to drive the uptake of cations and sugars into the vacuole via secondary transport (antiporter) systems.

Vacuolar membranes in addition to V-AT'Pases. contain another type of proton pump called H+ - Pyrophosphatase (H+-PPase). This pump consists of a single polypeptide with a molecular mass of 80 kDa. It is driven by the energy obtained from the hydrolysis of inorganic pyrophosphate (PPi). It is a inducible pump and is induced by low O_2 levels (hypoxia) or by chilling. Under these conditions ATP levels are depleted resulting in an inactivation of V-ATPase. In order to maintain 'essential cell activities under these stress conditions cells operate H+-PPase to regulate the ionic traffic and metabolites between the cytosol and the vacuolar sap across the tonoplast membrane.

Tonoplasts also contain still another large group of active transport proteins known as the ATP - binding cassette (ABC) transporters for the transport of large organic molecules into the vacuole. They are energized directly by ATP hydrolysis to pump organic molecules across a membrane. This family of active transport membrane proteins are divided into two main subclasses: the multidrug resistance proteins (MDRs) and the multidrug resistance - associated proteins (MRPs). Both types of proteins have been identified in plants, but only the MRPs were studied in detail.

Plants are also called glutathione conjugate pumps or GS-X pumps, because the molecules that are transported by these pumps are covalently attached to the tripeptide glutathione. The GS-X pumps of plant cells are specifically localized on the vacuolar membrane where they function in herbicide detoxification, protection against oxidative damage, pigment accumulation, and the storage of antimicrobial compounds. A family of enzymes called glutathione transferases (GSTs) are responsible for attaching glutathione to the organic molecule to be transported. Compounds such as anthocyanins, IAA, various phenolic compounds and phytochelatins after their attachment to the glutathione are transported into the vacuole by GS-X pumps.

4.7 SUMMARY

Molecular movement between different compartments of biological systems is known as transport. Plants exchange solutes with their environment and among their tissues and organs. Transport between cells is specifically controlled by their plasma membranes. Solutes may cross a membrane by simple diffusion, facilitated diffusion or active transport. In simple diffusion and facilitated diffusion transport of solutes occur down a chemical gradient called passive transport. Movement of solutes against a chemical potential gradient is known as active transport.

Facilitated diffusion involves channels as solute transporters while the active transport is mediated by membrane spanning pumps and carrier proteins: Only active transport achieves accumulation of ions against an electrochemical gradient. It requires a source of metabolic energy, normally in the form ofATP transport. Because ions carry an electrical charge so that they will diffuse in response to a gradient in electrical potential as well as chemical potential. That is for ions acted upon by an electrical gradient, cations are attracted to a negative electro potential whereas anions are attracted to a positive electropotential. Ion movement is thus dependent on an electrochemical potential gradient and the electrical potential of the cell or its transmembrane potential.

Generally living cells are negatively charged as compared with the outer medium. This is because the cytosol contains a large number of fixed or nondiffusible charges such as the carboxyl (RCOO-) and amino ($R.NH_4+$) groups of proteins. At the same time, cells use metabolic energy to actively pump cations like H+, Ca2+ and Na+ into the exterior space. As a result, a voltage or potential difference will develop across a membrane due to this unequal distribution of cations and anions. For this reason, the passage of ions through the plasma membrane or tonoplast must be considered in relation to the prevailing electrical potential gradient as well as the concentration gradient between the outer solution (medium) and inner solution (cytoplasm). Positively charged potassium ions; for example, will naturally be attracted to a region with a preponderance of negative charges.

The relationship between transmembrane potential gradient and ion. distribution across the

membrane can be expressed quantitatively by the Nernst equation:

$$\Delta Enj = \frac{2.3\ RT}{zF} \times \log \frac{C_j^i}{C_j^o}$$

Where ΔEn = the electrical potential difference for the ion j
C_j' / C_j'' = the ratio of the molar concentrations inside and outside the cell J J
R = gas constant
F = Faraday constant (96,500J V^{-1} mole-1)
z = Valency or charge for ion j. The value of z for a univalent cation would be 1 while for calcium or magnesium it would be 2. For chloride or nitrate it would be -1 and for sulphate it would be-2.

This equation allows us to say whether the ion is transported passively or actively. across the membrane. To apply the equation, it is necessary to first measure the transmembrane potential and the concentration of ions both inside and outside the cell. Deviations from the concentrations predicted by the Nernst equation are considered evidence that either active uptake or expulsion of the ions is involved. If the measured internal concentrations are approximately equal to the calculated' Nernst value, it can be assumed that the ion has been distributed passively. If the measured concentration is greater than predicted, active uptake is probably involved, and, if lower, the ion is actively expelled from the cell (Table 4.1).

Table 4.1 The uptake of selected ions by maize roots

Accumulation ratio			
Ion	**C^o(m)**	**C^i(m)**	**[C^i/C^o]**
K^+	0.14	160	1142
Na^+	0.51	0.6	1.18
NO_3^-	0.13	38	292
SO_4^{2-}	0.61	14	23

C^O and C^i are the ion concentrations of the medium and root tissue respectively.

4.8 MODEL QUESTIONS

1. Describe the mechanism of nutrient uptake by active transport process.
2. What are membrane transport proteins and what role do they play in nutrient uptake?
3. Write short notes on :
   a) Accumulation ratio
   b) Apoplast and symplast
   c) ABC transporter proteins

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Dr CVS Bhaskar

LESSON - 5

TRANSPIRATION - LOSS OF WATER'

OBJECTIVE:

In this lesson the process of transpiration, the structure of stomata and mechanismsfor stomatal opening and closure, and the role of vapor pressure differences in directing theexchange of-water between leaves and atmosphere have been discussed.

STRUCTURE OF THE LESSON:

5.1 INTRODUCTION

Plants absorb large quantities of water from the soil and translocate to the various parts of the plant. Of all the water absorbed by the plant, it retains less than five percent to maintain growth and even less is used biochemically. The balance passes through the plant to be lost as water vapor, a phenomenon known as transpiration. The quantitative importance of transpiration has been indicated by a variety of studies over the years. In his classic 1938 physiology book E.C. Miller revealed that a single maize plant might transpire as much as 200 liters of water over its lifetime and this transportational water loss is approximately 100 times its own body weight. Similarly, a single, 7.5 meter open-grown *Silver maple* tree may lose as much as 225 liters of water per hour. Whether there is any positive advantage to be gained by transpiration is a point of discussion, but the potential for such massive amounts of water loss clearly has profound implications for growth, development, productivity and even survival of plants.

5.2 THE PROCESS OF TRANSPIRATION

Transpiration is an inevitable phenomenon in which large amounts of water from the plant are lost in the form of water vapor to the atmosphere. From the vascular termini water moves to the leaf parenchyma, from which evaporation and loss of water takes place. There are two

pathways for this movement of water to the atmosphere. They are cuticular and stomatal and are strongly tied to leaf anatomy. The outer surfaces of a typical vascular plant leaf are covered with multilayered waxy deposit called the cuticle. The principal component of the cuticle is cutin, which is a heterogenous polymer of long chain 16 or 18 carbons containing hydroxylated fatty acids. Cutin forms an extensive polymeric network that in association with cuticular waxes, which are mixtures of long chain saturated hydrocarbons, alcohols, aldehydes and ketones, forms multilayered thickening on the leaf surfaces.

The cutin network is embedded in a matrix of cuticular **waxes**, which are complex mixtures of long-chainn(up to 37 carbon atoms) saturated hydrocarbons, alcohols, aldehydes, and ketones. Because cuticular waxes are very hydrophobic, they offer extremely high resistance to diffusion of both liquid water and water vapor from the underlying cells. The cuticle thus serves to restrict evaporation of water directly from the outer surfaces of leaf epidermal cells and protects both the epidermal and underlying mesophyll cells from potentially lethal desiccation.

The integrity of the epidermis and the overlying cuticle is occasionally interrupted by small pores called **stomata** (sing. **stoma**). Each pore is surrounded by a pair of specialized cells, called **guard cells**. These guard cells function as hydraulically operated valves that control the size of the pore . The interior of the leaf is comprised of photosynthetic **mesophyll** cells. The somewhat loose arrangement of mesophyll

This layer offers extremely high resistance to diffusion of both liquid water and water vapor from the underlying cells. Though it is meant to check transpiration, it is rarely completely impermeable due to some cracks in it. the more so when the layer is thin. So, a small amount of water absorbed may be lost from the underlying mesophyll cells to the bulk air through the cuticle. This is known as cuticular transpiration. The major pathway involves movement of liquid water from the leaf parenchyma in the form of vapor, to the air filled intercellular spaces and from there to the atmosphere through the stomatal pores in the epidermis. This is known as stomatal transpiration and this process accounts for 90 to 95 percent of the water loss from the leaves. Sometimes, loss of water also takes place through the lenticels of stems and fruits, and this is called lenticular transpiration. Both cuticular and lenticular transportational processes account for 5 to 10 percent of the water loss and is insignificant when compared to the amount of water lost by stomatal transpiration.

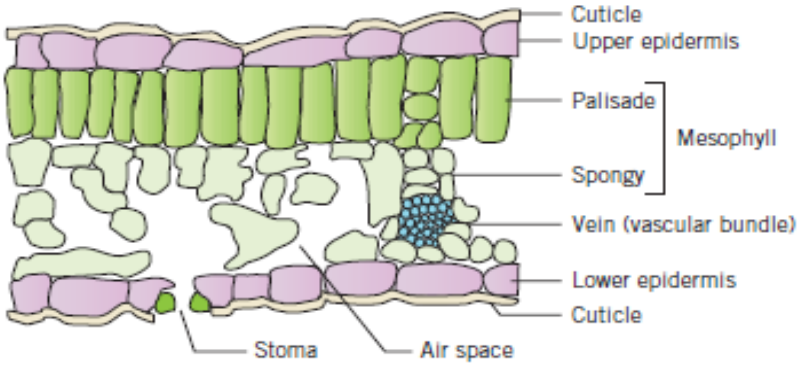

FIGURE 5.1 Diagrammatic representation of a typical mesomorphic leaf (*Acer* sp.) shown in cross-section. Note especially the presence of a cuticle covering the outer surfaces of both the upper and lower epidermis. Note also the extensive intercellular spaces with access to the ambient air through the open stomata.

5.3 STOMATAL TRANSPIRATION

Diffusion of water vapor through occasional interruptions of epidermis and the overlying cuticle, called the stomata (sing- stoma), is known as stomatal transpiration.

5.3.1 Structure of stomata

Stomata are minute pores in the epidermis of leaves and evolved for the exchange of gases between the internal air spaces and the ambient atmosphere. They are found in the leaves of virtually a" higher plants (angiosperms and gymnosperms) and most lower plants (mosses and ferns) except for submerged aquatic plants and liverworts. In higher plants there are fauna on most aerial parts including nonleafy structures, such as floral parts and stems, although they may be nonfunctional in some cases. The frequency and distribution of stomata is quite variable and depends on several factors including species, leaf position, ploidy level and growth conditions. A frequency (number of stomata per unit leaf area) in the range of 20 to 400 stomata mm^2 of leaf surface is quite common. In some cases, frequencies of 1000 mm^2 or more have been reported. The leaves of herbaceous monocots such as grass usually contain stomata on both the upper*(adaxial)* and lower (abaxial)with roughly equal frequencies. The herbaceous dicots also contain stomata on both sides of their leaves, but the frequency is usually lower on the upper surface. Most woody dicots and tree species have. stomata only on the lower leaf surface while floating *leaves* of aquatic plants (Le water lily) have stomata only on the upper surface.

The opening or pore of a stoma is surrounded by a pair of unique *cells* called guard cells. In most cases the guard cells are in turn surrounded by specialized, differentiated epidermal' cells called subsidiary cells. The opening together with its bordering guard cells and subsidiary cells is called the stomatal complex. The distinguishing feature of the stomatal complex is the pair of guard cells that function as a hydraulically operated value. Guard cells take up water and swell to open the pore when CO_2 is required for photosynthesis and lose water to close the pore when CO_2 is not required or when water stress overrides the photosynthetic needs of the plant. That is they have the capacity to undergo reversible turgor changes, that in turn regulate size of the pore between them.

Anatomically there are two basic types of guard cells: the gramineous type and elliptic type (Figure 5.1).

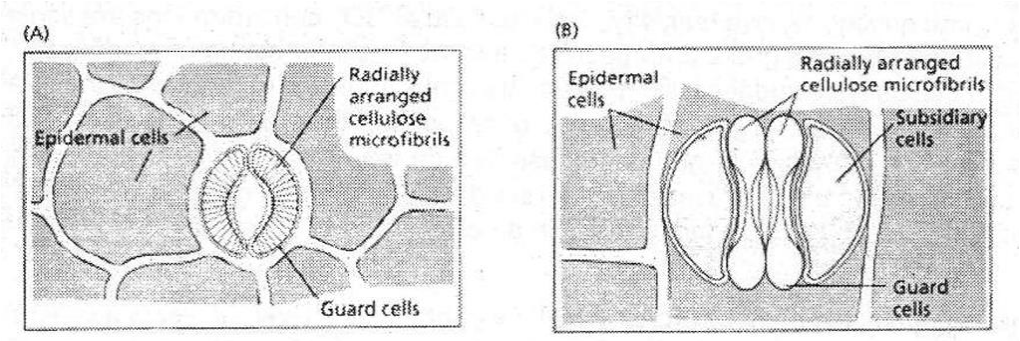

Figure 5.2 The radial alignment of the cellulose microfibrils in guard cells of(A) a kidney shaped stoma and (B) a graminacious type of stoma.

The graminaceous type of guard cells are largely restricted to members of the Gramineae and a few other monocots such as palms. These guard cells have a characteristic dumbbell. shape, with bulbous ends. The proper pore is a long slit located between the two 'handles' of the dumbbells. These guard cells have thickened cell walls toward the lumen and are always flanked by a pair of differentiated epidermal cells called subsidiary cells. In grasses, the dumbbell shaped guard cells function like beams with inflatable ends. As the bulbous ends of the cells increase in volume and swell, the beams (handles) are separated from each other and the slit between them widens (Figure 5.2 B).

Elliptic or kidney shaped guard cells are so called because of the elliptic shape of the opening. In surface view. these guard cells resemble a pair of kidney beans with their concave sides opposed, in cross section the cells are roughly circular in' shape, with a ventral wall bordering the pit and a dorsal wall adjacent to the surrounding epidermal cells. The mature guard cells have characteristic wall thickenings that cause guard cells to become concave during stomatal opening. This thickening pattern is associated with the alignment of their cellulose microfibrils. In ordinary cells with a cylindrical shape cellulose microfibrils are oriented to transverse the long axis of the cell. As a result, the cell expands in the direction of its long axis, since the cellulose reinforcement offers the least resistance at right angles to its orientation (Figure 5.1A). In guard cells the microfibril organization is different where the cellulose microfibrils 'fanning out radially from the pore. Thus, the cell girth is reinforced like a steel-belted radial tire, and the guard cells curve outward, during stomatal opening.

5.3.2 Mechanism of stomatal movements

More than 90 percent of the CO_2 and water vapor exchanged between a plant and its environment passes through the stomata. Stomata are therefore involved in controlling two very important but competing processes such as uptake of CO_2 for photosynthesis from the atmosphere and transpirational water loss to the atmosphere. So, it is important to discuss the stomatal functioning in view of the photosynthetic productivity and crop yields of higher plants.

Guard cells function as multisensory hydraulic valves. Environmental factors such as light intensity and quality, relative humidity, and intercellular CO_2 concentrations are sensed by guard cells, and these signals are integrated into well-defined stomatal responses. If leaves kept in the dark are suddenly illuminated, the light stimulus is perceived by the guard cells as an opening signal, triggering a series of responses that result in opening of the stomatal pore. The early events of this process include ion uptake, decrease in osmotic potential, and osmotic uptake of water by the guard cells and the consequent increase in hydrostatic pressure. These changes result in a deformation of guard cells with wide pore.

What regulates the osmotic properties of the guard cells? Over the years: a variety of mechanisms have been proposed to explain osmotic concentrations of guard cells. The botanist H. Von Mohl proposed in 1856 that turgor changes in guard cells provide the driving force for stomatal movements, and the plant physiologist F.E. Leoyd hypothesized in1908 that these turgor changes depend on starch-sugar interconversions, a concept that led to a starch-sugar hypothesis of stomatal movements. Guard cell chloroplasts contain large, prominent starch grains and their starch content decreases during stomatal opening and increases during closing. Starch, an insoluble glucose polymer, does not contribute to the cell's osmotic potential, but the hydrolysis of starch into soluble sugars causes an increase in

the osmotic potential of guard cells. In the reverse process starch synthesis decreases sugar concentration, resulting in a lowering of the cell's osmotic potential with associated stomatal closing.

This hypothesis was widely accepted until the discovery of potassium fluxes in guard cells by S. Imamura in 1943, which were later confirmed by M.Fujino and R.A. Fischer. In the late 1960s it became evident that potassium levels are very high in open guard cells and very low in closed guard cells. Potassium concentrations can increase severalfold in open stomata, from 100 mM in the closed state to 400 to 800 mM in the open state, depending on the plant species and the experimental conditions. An accumulation of K+ in guard cells is now accepted as a universal mechanism in stomatal opening (Figure 5.2):

The accumulation of ions by most plant cells is driven by the proton-pumping H+-ATPase located on the plasma membrane. Proton pumping by an ATP driven H+-ATPase, which is one of the initial events in stomatal opening, is evidenced from several lines of research. First, the fungal toxin fusicoccin, which is known to stimulate *active* proton pumping' from' the inside to outside of the cell by the H+-ATPase, stimulates stomatal opening Second, *vandate* (VO_3-) which inactivates the proton pump, and carboxyl cyanide chlorophenylhydrazone (CCCP) which abolishes H+-ATPase generated proton gradient, inhibits stomatal opening. Thus, proton extrusion is the initial event that causes a voltage of potential difference across the membrane because of unequal distribution of anionic and cationic charges. In addition, proton pumping also generates a pH gradient of about 0.5 to 1pH unit. Energy stored in the resulting electrochemical proton gradient, also known as the proton motive force, provides a driving force for the passive uptake of potassium ions via Voltage-regulated potassium channels (Figure 5.2).

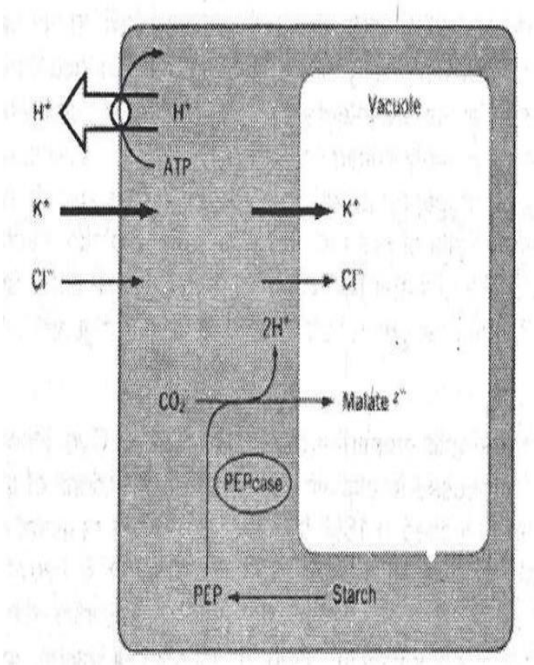

Figure 5.3 A proposed model for stomatal opening. 'Potassium uptake is driven by an ATPase - proton pump located in the plasma membrane. The accumulation of ions in the vacuole lowers the water potential of the guard cell, thereby stimulating the osmotic uptake of water and increased turgor.

To maintain electrical neutrality, excess K^+ion accumulated in the cells must be balanced by a counter ion carrying, a negative charge. According to the models in Figure 3.2, charge balance is achieved partly by an Influx of chloride ion (CI^-), and partly by organic anions such as malate. In species of the genus *Allium,* such as onion (*Allium cepa),*K^+ions are balanced by CI^-. In most species, however, potassium fluxes are balanced by varying amounts of $Cl^-$and the organic anion malate $^{2-}$.

Like potassium, chloride is taken up into the-guard cells during stomatal opening and extruded during stomatal closing. It is thought to be taken up through a CI^- - H^+ symporter. Malate, on the other hand, is synthesized in the guard cell cytosol, in a metabolic pathway that uses carbon skeletons generated by starch hydrolysis. The malate content of the guard cells decreases during stomatal closing.

The accumulation of K^+CI^- and malate in the vacuoles of the guard cells would lower the osmotic potential. As osmotic potential decreases, the water potential also decreases, and water consequently moves into the guard cells. As water enters the cell, turgor pressure increases, which in turn causes reversible deformation of the guard cells to open the stomata. At present this remains a working model for stomata opening since many of the details have yet to be experimentally verified.

Stomatal closure has not received the much attention as that of opening. Its closure is affected simply by a reversal of events leading to stomatal opening. According to the presently available information, signals for stomatal closure stimulate the uptake of Ca^{2+} into the cytosol. Ca^{2+} uptake, thus initiating a chain of signal transduction events that includes opening anion channels to allow release of CI^- and malate^{2-}. A loss of these anions then depolarizes the membrane. Membrane depolarization is accompanied by the passive diffusion of potassium ions into the adjacent subsidiary and epidermal cells through opened K+channels.

When coming to the source of ATP to drive an ATPase proton pump in guard cells, it seems that ATP is generated either by photosynthesis in those guard cells that contain Chloroplasts or from carbon oxidation through normal respiratory pathways. Stomatal closure also occurs in response to water stress. This kind of stomatal movement is called hydro active closure, which takes place when the plant senses a water deficit and initiates a specific mechanism to induce closure. The mechanism for hydro active closure involves the same ion fluxes normally associated with closure but is triggered by water deficit in the leaf and is mediated by the hormone abscisic acid (ABA).

ABA is a normal constituent of leaves, where it is synthesized at low rates in unstressed mesophyll cells and accumulates in the chloroplasts. In an actively photosynthesizing leaf, the pH of the chloroplast stroma is normally higher (pH 7.5 to 8.0*)* than that of the cytosol (pH 7.0 to 7.5). This pH difference leads to a large accumulation of ABA in the chloroplast. Moderate water stress causes a decrease in the pH of the chloroplast stroma and an increase in the cytosolic and apoplastic pH. Such changes in pH then causes the release of ABA from the chloroplast stroma into the apoplastic space. ABA from apoplastic space is carried to the guard cells through the transpiration stream and initiates stomatal closure. Because of its ability to stimulate stomatal closure and thus reduce transpirational water loss, ABA has been referred to as an "anti-transparent or stress hormone". We will now examine the driving force for leaf transpiration.

5.4 THE DRIVING FORCE FOR TRANSPIRATION

The driving force for water loss is difference in water vapor concentration. The difference in water vapor concentration is expressed as C_{WV}(leaf)- Cwv (air). According to Fick's law of diffusion, molecules will diffuse from a region of high concentration to a region of *low* concentration, or, down a concentration gradient. Vapor pressure is proportional to vapor concentrations that water vapor will also diffuse down a vapor pressure gradient; that is, from a region of high vapor pressure to a region of lower vapor pressure, Stomata are located such that, when open, they provide a path for the movement of water vapor between the internal air space and the bulk atmosphere surrounding the leaf. Because of this relationship, this space is referred to as substomatal space. This substomatal air space of a leaf is normally saturated or very nearly saturated with water vapor. This is because the mesophyll cells which border the air space present a large, exposed surface area for evaporation of water. On the other hand, the atmosphere which surrounds the leaf is usually unsaturated and may often have a very low water content. These circumstances create a gradient between the high water vapor pressure in the substomatal air space of the leaf and lower water vapor pressure of the external atmosphere. This difference in water vapor pressure drives the movement of water vapor molecules from the internal air spaces of the leaf to the surrounding bulk air.

water movement is determined by differences in water potential. It then 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 vapor rather than liquid water, it will be more convenient to think in terms of vapor systems. Consider what happens, for example, when a volume of pure water is introduced into a closed chamber (Figure 5.2). Initially the more energetic water molecules will escape into the air space, filling that space with water vapor. Some of those water molecules will then begin to condense into the liquid phase. Eventually water in the chamber will reach a dynamic equilibrium; the rate of evaporation will be balanced by the rate of condensation.

The air space will then contain the maximum amount of water vapor that it can hold at that temperature. In other words, *at equilibrium the gas phase will be saturated with water vapor*. The concentration of water molecules in a apor phase may be expressed as the vapor mass per unit volume (g m−3), called **vapor density**. Alternatively, the concentration may be expressed in terms of the pressure exerted by the water vapor molecules against the fluid surface and walls of the chamber. This is called **vapor pressure** (symbol = *e*). With an appropriate equation, vapor density and vapor pressure are interconvertible.However, because we are now accustomed to dealing with the components of water potential in pressure units, it will be more consistent for us to use vapor pressure (expressed as kilopascals, kPa) in our discussion. We can then say that when a gas phase has reached equilibrium and is saturated with water vapor, the system will have chieved its **saturation vapor pressure**. The vapor pressure over a solution at atmospheric pressure is influenced by both solute concentration and temperature. As was previously discussed with respect to water potential (Chapter 1), the effect of solute concentration on vapor pressure may be expressed in terms of the mole fraction of water molecules. This relationship is given by a form of Raoult's law, which states:

$$e = Xi\ eo \quad (2.1)$$

where *e* is vapor pressure of the solution, Xi is the mole fraction of water (=number of water molecules/number of water molecules + number of solute molecules), and *e*o is the saturation vapor pressure over pure solvent. The actual reduction in vapor pressure due to

solute turns out to be quite small. This is because even in elatively concentrated solutions the mole fraction of solvent remains large. Conider, for example, a 0.5 molal solution, which is approximately the concentration of vacuolar sap in a typical plant cell. A 0.5 molal solution

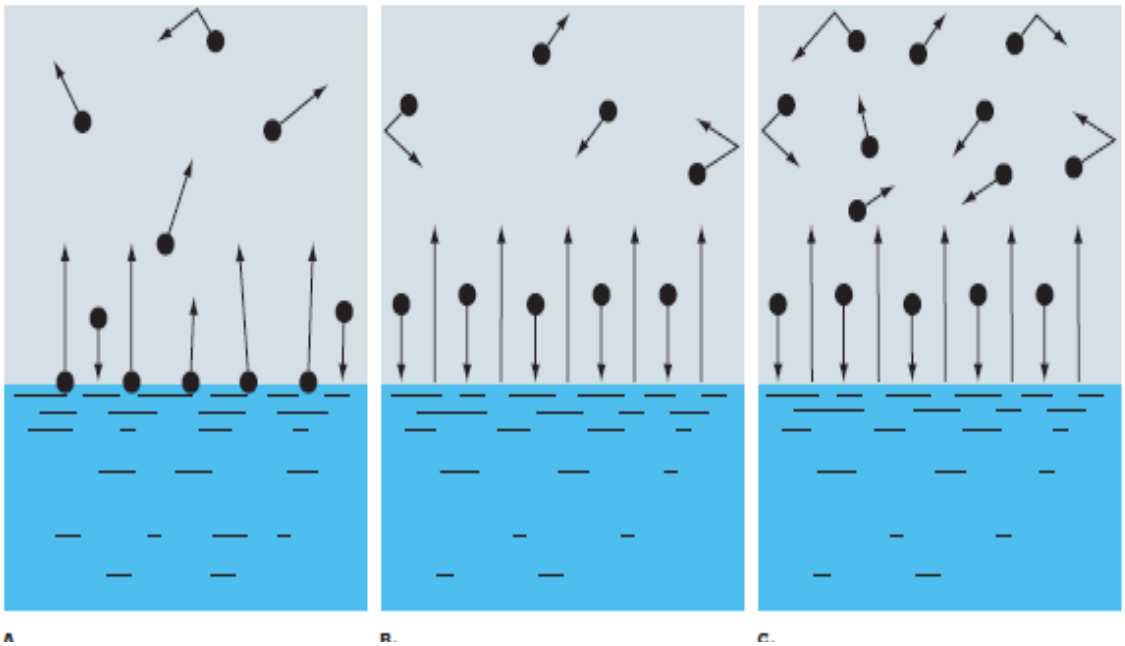

FIGURE 5.4 Vapor pressure in a closed container. Initially (*A*), more molecules escape from the water surface than condense, filling the air space with water vapor molecules. The vaporous water molecules exert pressure—vapor pressure—against the walls of the chamber and the water surface. At equilibrium (*B*) the rate of condensation equals evaporation and the air is saturated with water vapor. The vapor pressure when the air is saturated is known as the saturation vapor pressure. At higher temperature (*C*), a higher proportion of water molecules have sufficient energy to
escape. Both the concentration of water molecules in the vapor phase and the saturation vapor pressure are correspondingly higher.

5.5 TRANSPIRATION RATIO

The effectiveness of plants in regulating water loss while allowing sufficient CO_2 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 ofCO_2 assimilated by photosynthesis.

$$\text{Transpiration ration} = \frac{\text{Moles of } H_20 \text{ transpired}}{\text{Moles of } CO_2 \text{ fixed}}$$

For a typical plant in which the first stable product of carbon fixation is a three-carbon compound, about 500 molecules of water are lost for every molecule of CO_2 fixed by photosynthesis, giving a transpiration ratio of 500. Sometimes the reciprocal of the transpiration ratio, called the water use efficiency, is cited. Plants with a transpiration ratio of 500 have water use efficiency of 1/500, or 0.002.

The large ratio of H_20 efflux to CO_2 influx depends on two factors. First, the concentration gradient driving water loss is 50 times larger than that of the driving CO_2 influx. In large part,

this difference is due to the low concentration of CO_2 in air (about 0.03%) and the relatively high concentration of water vapor within the leaf air spaces. Second, CO_2 diffuses about 1:6times more slowly through air than water does (the CO_2 is larger than H_2O and hence has a smaller diffusion coefficient).

5.6 SIGNIFICANCE OF TRANSPIRATION

Transpiration has got immense significance in plant life as it is of great benefit to the plant. Though a large amount of absorbed water is lost during transpiration, which is no doubt a harmful effect.

The theory of evolution states that any harmful feature should be eliminated by natural selection. But transpiration is obvious in any land plant. So, it is definitely advantageous despite its harmful features or in other words, the benefit is much greater than the harm. For that reason, transpiration is often said to be a necessary evil, The advantage of transpiration is a sort of 'victory by default'.

Transpiration is not only beneficial but also essential in the life of land plants for the absorption of CO_2required for photosynthesis: Besides the gaseous exchange, it gives cooling effect.by regulating the temperature in the leaves of terrestrial plants that are often exposed to intense sun light and provides a driving force for the ascent-of sap from ground level to the, top of the tallest trees.

5.7 SUMMARY

Transpiration is an inevitable phenomenon in which large amounts of water are continuously lost in the form of vapor to the surrounding atmosphere from the surfaces-of aerial parts because of their structural organization. There are three types of transpiration: stomatal, cuticular, and lenticular. The major portion of water (90 to 95%) is transpired through stomata; the mechanism of transpiration is mainly dependent on the mechanism of stomata opening and closing. The stomatal movement depends on the increase or decrease in the osmotic potential of the guard cells. These osmotic changes result in changes in water potential causing movement of water in and out of the guard cells, according to the current hypothesis, the osmotic potential of guard cell and consequently, the size of the stomatal opening, is determined by the extent of K- accumulation in the guard cells. This theory, also known as proton transport concept, can 'explain the obvious facts occurring during stomatal opening namely (a) excretion of $H^+$from guard cells, (b) uptake of K^+ into the guard cell vacuole, (c) uptake of CI' into the vacuole and (d) production of organic acid, particularly malate, Stomatal closure involves several events leading to opening. Under conditions of water deficit, the ABA acts as an antitranspirant and closes the stomata, The gradient in the water vapor concentration between the internal air spaces of the leaf and the surrounding atmosphere, is the driving force of transpiration.

5.8 MODEL QUESTIONS

1. Explain how guard cells regulate the size of the stomatal aperture
2. Write short notes on :
   a) Transpiration ratio
   b) Structure of stomata
   c) Role of ABA in the mechanism of stomatal closure

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LESSON- 6

ESSENTIAL NUTRIENTS, DEFICIENCIES AND PLANT DISORDERS

OBJECTIVE :

In this lesson, the criteria for essentiality of mineral nutrients, classification of nutrients based on their relative requirement and biological functions, mineral nutritional studies through various techniques and nutrient roles and their deficiency symptoms are discussed.

STRUCTURE OF THE LESSON:

6.1 INTRODUCTION

Plants must have a supply of raw materials from the environment to obtain energy and construct new cellular components. The supply and absorption of chemical compounds needed to maintain their metabolism, growth and development may be defined as **nutrition.** The chemical compounds required by an organism are termed as **nutrients.** Plants absorb these chemical elements through their roots principally as inorganic ions from the soil. The study of how plants absorb and assimilate these inorganic ions is called **mineral nutrition.** This area of plant research has now been playing an important position in designing modem agriculture and environmental protection. Much of the groundwork for modem nutritional studies was aimed with an interest to get high agricultural yields. In this context, we must give first credit to French plant physiologist **"de** Saussure", In 1804 de Saussure clearly demonstrated that the inorganic mineral elements contained in the ash of plants are obtained from the soil via the root system. He suggested that some of the chemical elements found might be indispensable (i.e., essential) to plant growth. Serious study of the identity of mineral nutrients required for plant growth was then began by others who followed de

Saussure. C.S.Sprengel (1787-1859) working in Germany had of the opinion that soil might be unproductive ifit is deficient even in single element necessary for growth. Further, the French agronomist Boussingault stressed about the quantitative relationships between the effects of fertilizer and nutrient uptake in crop yield. By the middle of the 19th century, the' technique of growing plants-in defined nutrient solutions in the complete absence of soil has been established particularly through the efforts of the two German plant physiologists, Sachs and Knop (1860). Finally, J.B. Lowers and R.N.Gilbert working in England, had successfully converted insoluble rock phosphate to soluble phosphate called super phosphate. By the end of the century the use of nitrogen, phosphorous and potassium (N-P-K) fertilizers in agriculture was well established. According to one estimate, the use of these fertilizers in agriculture rose steadily from 112 million metric tons in 1980 to 143 million metric tons in 1990.

Based on nutritional requirements for carbon containing compounds, organisms have been classified as **autotrophs** and **heterotrophs.** Plants, most algae and a few bacteria are **autotrophic**(self-nourishing) organisms. They live in an entirely inorganic environment, taking' in CO_2 from the atmosphere and water and mineral nutrients from the soil. In contrast to autotrophs, most bacteria, fungi and all members of the animal kingdom depend for their existence on energy-rich organic molecules previously synthesized by autotrophs. Hence, they are called heterotrophs. The autotrophic ability of the green plants has a key role in nature because that provides continuous cycling of inorganic ions between organisms and their environment. Plants having this ability of absorbing inorganic nutrients from the soil solution through their large-surface area of roots made Epstein (1972,1974) call them **"miners"** of earth's crust.

6.2 NUTRIENT SOURCES

The nutrients indispensable for growth and development of green plants is derived from three environmental sources, the atmosphere, water and the soil. The atmosphere furnishes CO_2 andO_2. Water is a second source of nutrition that gives hydrogen atoms and O_2, Carbon and most of theO_2derived from CO_2 (also from the H_2O) together with hydrogen make up 90% of the dry weight of the plant. Soil is a third environmental source of mineral nutrients. Mineral elements of soil are mainly derived from the parent rock' and from decaying plant and animal debris. Majority of the plants absorb mineral ions mostly by roots from the soil. However, certain species of higher plants that grow as epiphytes absorb minerals from airborne dust particles, which encounter their surfaces and then dissolve in dew or rainwater.

6.3 CRITERIA FOR ESSENTIALITY

The inorganic' chemical elements that have a clear physiological role in the growth and development of a plant and whose absence prevents a plant from completing its life cycle are defined as essential **elements.** Analysis of plants reveals the presence of a' large number of mineral elements. Infact, 'all dements found in a plant are not essential for its growth and development. Many of them are non-essential. To find out the essentiality of an element for a plant, three criteria have been proposed by Amon arid Stout (1939) and Epstein (1972).

1. A, deficiency of the element makes it impossible for the plant to complete its life cycle.
2. The deficiency is specific to the element in question. It cannot be replaced by any other element.

3. The element is directly 'involved in the nutrition of the plant and not causing some other element to be more readily available or antagonize the effect of another element.

These three requirements form the criteria for essentiality. According to the first criterion an element is said to be essential, if a plant is unable to produce viable seed in the absence of that element. By the second criterion the essential role of elements, for example Mg in chlorophyll and N in proteins, cannot be compensated by any other element. The third criterion has got less importance in deciding essentiality, but there are few cases in which it has been applicable. The growth promoting effects selenium, for example, resulted from the ability of the selenate ion to inhibits the absorption of the phosphate, which was otherwise absorbed by the plant's intoxicants. Some recent studies have shown that some of the essential elements. can be partially replaced by others, for example, magnesium by manganese, potassium by rubidium and chlorine by bromine.

Based on these' criteria, at present 17 chemical elements are recognized to be essential for the growth of higher plants (Table 6.1)

TABLE 6.1 The essential nutrient elements of higher plants and their concentration considered adequate for normal growth'

Element	Chemical Symbol	Available Form	Concentration in Dry matter (m mol /kg
Macro nutrients			
Hydrogen	H	H_2O	60000
Carbon	C	CO_2	40000
Oxygen	O	O_2, CO_2	30000
Nitrogen	N	NO_3^-, NH_4^+	1000
Potassium	K	K^+	250
Calcium	Ca	Ca^{2+}	125
Magnesium	Mg	Mg^{2+}	80
Phosphorous	P	HPO_4^-, HPO_4^{2-}	60
Sulfur	S	SO_4^{2-}	30
Micronutrients			
Chlorine	Cl	Cl^-	3.0
Boron	B	BO_3^{3-}	2.0
Iron	Fe	Fe^{2+},Fe^{3+}	2.0
Manganese	Mn	Mn^{2+}	1.0
Zinc	Zn	Zn^{2+}	0.3
Copper	Cu	Cu^{2+}	0.1
Nickel	Ni	Ni^{2+}	0.05
Molybdenum	Mo	Mo_4^{2-}_	0.001

6.4 MACRO AND MICRONUTRIENTS

The seventeen essential mineral elements as shown in table 6.1 are divided into macronutrients and micronutrients. Such a distinction was proposed according to their relative concentrations found in plant tissue or required in nutrient solutions. It is difficult to justify the classification of plant nutrients into macronutrients and micronutrients depending

on element concentration in plant tissues. Mengel and Kirkby (1987) therefore proposed an alternative and more meaningful system for nutrient division. They divided essential plant nutrients into four basic groups based on their biochemical behavior and physiological function (Table 6.2).

Table 6.2 Classification of Plant Mineral nutrients according to their chemical Function

Nutrient Elements	Functions
Group 1	Nutrients that form the organic compounds of Plants
N	Constituents of amino acids, amides, proteins, Nucleic acids, coenzymes
S	Components of cysteine, cysteine and methionine, proteins, constituents of lipoic acids, coenzyme A, thiamine, pyrophosphate, glutathione, biotin, adenosine 5' phosphorsulfates etc.
Group 2	Nutrients that are important in energy storage or structural integrity
P	Components of Sugar phosphates, nucleic acids, nucleotides, Coenzymes, phospholipids, phytic etc. has a key role in reactions in which ATP is involved
B	Complexes with mannitol, mannan, polymannuronic acid, and other constituents of cell wall involved in cell elongation and nucleic acid metabolism
Si	Deposited as amorphous silica in cell walls, contributes to cell walls, mechanical properties, including turgidity and elasticity
Group 3	Nutrients that remain in ionic forms
K	Required as cofactor for more than 40 enzymes, Principal cation in establishing cell turgor and maintaining cell electron neutrality
Na	Involved with the regeneration of phosphoenolpyruvate in c4 and CAM plants. substitute for potassium in some functions
Mg	Required by many enzymes involved in phosphate transfer. Constituents of the chlorophyll molecule
Ca	Constitutient of the middle lamellae of cell wall. Required as a cofactor by some enzymes involved in the hydrolysis of ATP and phospholipids. Acts as a second messenger in metabolic regulation
Mn	Required for the activity of some dehydrogenases, decarboxylases, oxidases, and with other cation activated enzymes and photosynthetic O_2 evolution
Cl	Required for the photosynthetic reactions involved in O_2 evolution
Group 4	Nutrients that are involved in electron transfers
Fe	Constituents of cytochrome and nonheme iron proteins involved in photosynthesis N2 fixation, respiration
Cu	Components of ascorbic acid oxidases, tyrosinase, phenolase and plastocyanin
Zn	Constituents of glutamic dehydrogenase, carbonic anhydrases
Mo	Constituents of nitrogenases, nitrate reductase
Ni	Constituents of urease in N2 fixing bacteria etc.

The first group includes the elements such as C,H,O,N and S that form the organic compounds of the plant. Plants assimilate these elements through biochemical reactions involving oxidation and reduction.

Phosphorous, boron and silicon constitute second group of elements. They are important in energy transfer reactions or in maintaining structural integrity. They are often present in plant

tissues as phosphate, borate, and silicate esters in which the elemental group is bound to hydroxyl groups of sugars.

The third group of plant nutrients is made up of K, Na, Mg, Ca, Mn and Cl. In the plant cell they are present in the free ionic state or are adsorbed in diffusible organic anions, for example, carboxylic groups of the pectin. They are important as cofactors of enzymes and in the regulation of osmotic potentials.

Members of the fourth group includes Fe, Cu, Zn, Mo and Ni. These elements are important roles in reactions involving electron transfer.

Elements other than those given in Table 6.1 can also accumulate in plant tissues. For some Sodium (Na), silicon (Si), cobalt (Co), selenium (Se), and aluminum (AI) have now been established as an essential element. These elements are not required to all higher plants. Hence, they are referred to as beneficial elements rather than essential elements. All Chemical elements except C, H and a are mineral elements.

6.5 TECHNIQUES USED IN NUTRITIONAL STUDIES

Several methods have been proposed for the study of plant nutrition, but following methods are very common in use.

6.5.1Ashanalysis: In this method of elemental study, the plants are subject to high temperatures of about 600°C in a muffle furnace. At this temperature the organic materials will be oxidized and driven off as water and carbon dioxide. The small quantity of white or grey matter left as residue is called ash. It contains all the mineral elements that were absorbed from the soil. The mineral elements of this ash are determined even in micro and semi micro quantities by spectrophotometric, colorimetric, turbidimetric, flame photometric and titrimetric methods. This method is a crude one because in the ash mineral elements are not found in pure form but occur in the state of oxides.

6.5.2 Solution culture: This technique *is* used to understand the kinds and *amounts* of elements essential to plants. In solution culture technique, the plants are grown with their roots immersed in a nutrient solution containing only inorganic salts. This technique of growing plants in a defined nutrient solution without soil is called **hydroponics** (Greek hudor = water; ponos = working). Woodward(1699) for the first time used water culture technique. This method in real sense was worked out further by Sacks (1860), Knop (1865), and Arnon and Hoagland (1940). Nutrient solutions formulated by Sachs (1860) and Knop (1865) are given in table 6.3 awl 6.7. respectively.

Table 6.3 The composition of Sachs' nutrient solution

SALT	**FORMULA**	**APPROXIMATE CONCENTRATION (mM)**
Potassium nitrate	KNO_3	9.9
Calcium phosphate	$Ca_3(PO_4)_2$	1.6
Magnesium sulfate	$MgSO_47H_2O$	2.0
Calcium sulfate	$CaSO_4$	3.7
Sodium chloride	NaCl	4.3
Iron sulfate	$FeSO_4$	trace

Table 6.4 The composition of Knop's nutrient medium

SALT	FORMULA	APPROXIMATE CONCENTRATION (mM)
Calcium nitrate	Ca(NO,)	0.8
Potassium nitrate	KNO_3	0.2
Potassium dihydrogen phosphate	KH_2PO_4	0.2
Magnesium sulfate	$MgSO_47H_2O$	0.2
Iron sulfate	$FeS0_4$	traces

In the beginning it was thought that these nutrient solutions may contain all the minerals required by the plant. But these nutrient solutions used in their experiments have chemicals that were contaminated with other elements such as boron or molybdenum that are now known to be essential.

Compound	Molecular weight	Concentration of stock solution	Concentration of stock solution	Volume of stock solution per liter of final solution	Element	Final concentration of element	
	g mol^{-1}	m*M*	g L^{-1}	mL		μ*M*	ppm
Macronutrients							
KNO_3	101.10	1,000	101.10	6.0	N	16,000	224
$Ca(NO_3)_2 \cdot 4H_2O$	236.16	1,000	236.16	4.0	K	6,000	235
$NH_4H_2PO_4$	115.08	1,000	115.08	2.0	Ca	4,000	160
$MgSO_4 \cdot 7H_2O$	246.48	1,000	246.49	1.0	P	2,000	62
					S	1,000	32
					Mg	1,000	24
Micronutrients							
KCl	74.55	25	1.864	2.0	Cl	50	1.77
H_3BO_3	61.83	12.5	0.773		B	25	0.27
$MnSO_4 \cdot H_2O$	169.01	1.0	0.169		Mn	2.0	0.11
$ZnSO_4 \cdot 7H_2O$	287.54	1.0	0.288		Zn	2.0	0.13
$CuSO_4 \cdot 5H_2O$	249.68	0.25	0.062		Cu	0.5	0.03
H_2MoO_4 (85% MoO_3)	161.97	0.25	0.040		Mo	0.5	0.05
NaFeDTPA (10% Fe)	558.50	53.7	30.0	0.3–1.0	Fe	16.1–53.7	1.00–3.00
Optional[a]							
$NiSO_4 \cdot 6H_2O$	262.86	0.25	0.066	2.0	Ni	0.5	0.03
$Na_2SiO_3 \cdot 9H_2O$	284.20	1,000	284.20	1.0	Si	1,000	28

Source: After Epstein 1972.

Table 6.5 shows a more modem formulation for a nutrient solution. This nutrient medium is called modified Hoagland solution, named after D.R.Hoagland.

A modified Hoagland solution contains all the mineral elements needed for rapid plant growth. This nutrient medium is specialized in having higher concentrations of mineral elements than those found in the soil in addition to nitrogen source both as ammonium (NH_4^+) and nitrate (NO_3^-) and iron as ferric sodium ethylene diamine tetraaceticacid (NaFeEDTA) or diethylene triaminepenta acetic acid (NaFeDTPA). The high initial level of elements allows the plants to be grown in a medium for extended periods without replenishment of the nutrients. Supplying nitrogen in a balanced mixture of NH_4^+ (cation) and NO_3^- (anion) tends to reduce the rapid rise in medium pH. Generally, iron can precipitate out

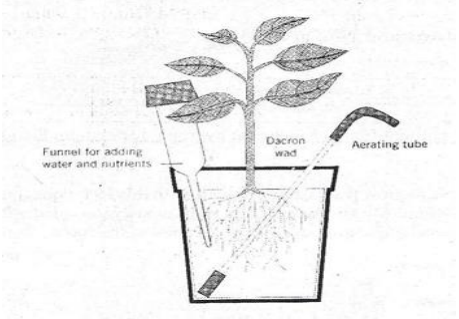
of solution as iron hydroxide when it is supplied in the form of Fe SO_4 or $Fe(NO_3)_2$. This problem can be eliminated by providing iron in the form of iron chelator (NaFeEDTA or NaFcDPTA) complex which is readily available to the plants when compared to iron supplied in the form of Fe S04 (or) Fe (N03)2to the nutrient solution
(Table 6.5).

Note: The macronutrients are added separately from stock solutions to prevent precipitation during the preparation of the nutrient solution. Acombined stock solution is made up containing all micronutrients except iron. Iron is added as sodium ferric diethylenetriaminepentaacetate(NaFeDTPA,trade name Ciba-Geigy Sequestrene 330).

...•• Nickel is usually present as a contaminant of the other chemicals, so it may not need to be added explicitly. Silicon. if included, should be added first and the pH adjusted with HCI to prevent precipitation of the other nutrients.
'.

In the simplest form of solution, culture a seedling is supported in the lid of a clean glass container containing dissolved mineral elements in double glass distilled water (Figure 6.1). The container in which the plants are grown is usually painted black or covered with an opaque material to protect the roots from direct light and to check algal growth in the medium. Aeration of the roots growing in the medium of a container is provided by aerating tube. This is to prevent development of anoxic conditions in nutrient solutions. Anoxia inhibits the respiration of root cells and reduces nutrient uptake. In this technique, to ascertain the essentiality of a particular mineral element to the growth and development of plants, every time only one element is left out from the solution, and the plant is grown on it. In the absence of that element if plant shows some deficiency symptoms, and if these symptoms disappeared on supplying the missing element, then that element is decided as an essential element.

Successful solutions or hydroponic culture require frequent replenishment of nutrient solutions to prevent ion depletion and associated changes in the P^H due to root absorption. To overcome this problem, some investigators grow the plants in a non-nutritive medium, such as acid washed quartz sand, perlite or vermiculite. Seedlings are raised on either of these solid media filled in container. Plants grown in this way can then be watered by daily application of fresh nutrient solutions. In this technique nutrient solution to the plants is provided three ways: (1) by pouring over solid medium(called slope-culture), (2) by dripping on to the solid medium at suitable intervals from a reservoir (drip culture) and (3) by pumping solution up from bottom of the container (sub-irrigated culture), This, fill and empty Process is repeated on a regular basis which serves both to replenish the nutrient solution and to aerate the roots. By eliminating one element at a time and by comparing the growth of the plant in its presence and absence, the essentiality of that element to the plant can be decided.



6.5.3 Nutrient film growth technique

An alternative hydroponic system that is often used at commercial level is called the nutrient film growth system. In this technique plants are grown in a tube or trough placed on a slight incline(Figure 6.2). The nutrient solution is pumped as a thin film from a reservoir to the elevated end of the tube by a pump. The solution then flows down the shallow trough surrounding the plant roots. This technique therefore allows the plant roots to be bathed continuously in a thin film of aerated nutrient solution. In this system, the composition and P^H of the nutrient solution can be controlled automatically.

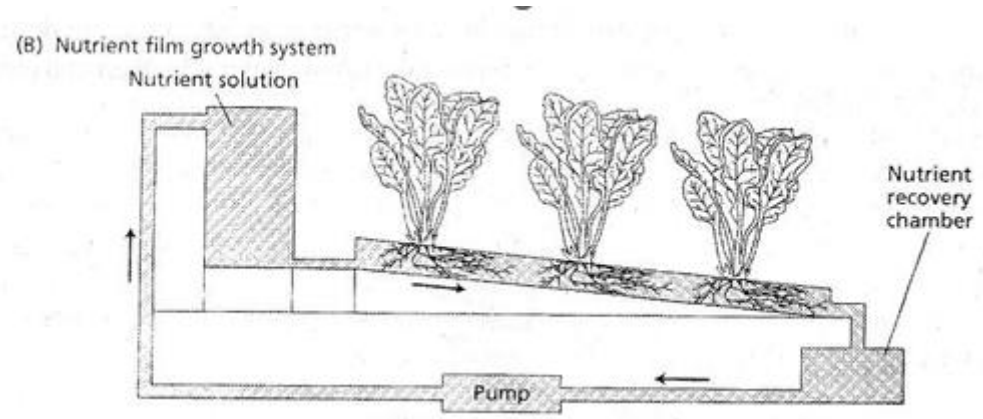


Figure 6.2 The nutrient film technique for hydroponic plant production

6.5.4 Aeroponics

Another alternative is to grow plants aeroponically. In this technique, plants are grown with their roots suspended in air (Figure 6.3). The roots are continuously provided with a nutrient mist of known concentration. This technique provides proper aeration to the roots. But at the same time, it requires more quantity of nutrient solutions than it is in hydroponic culture. For this reason and other technical difficulties, the use of aeroponics is not widespread. Recently, the techniques to measure concentrations of elements as low as 10.8 g ml^{-1} in plants, soils and nutrient solutions have been improved tremendously. These include the use of atomic

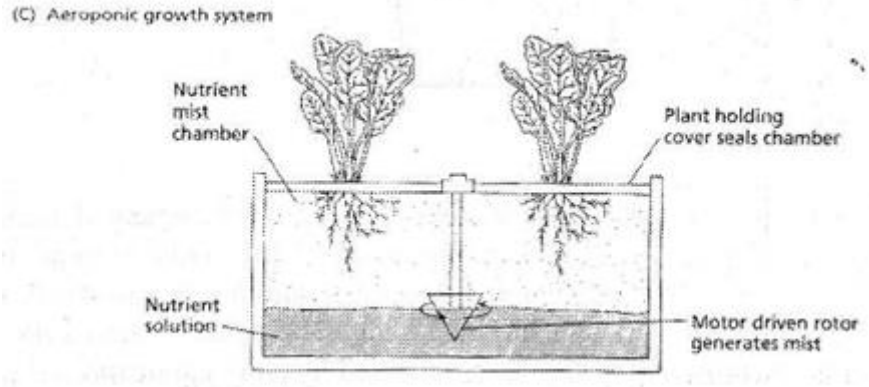


Figure 4.3 Diagram of a typical aeroponic system.

absorption spectrophotometers or atomic emission spectrometers. They are now used to measure mineral elements. These techniques require vaporization of the elements at temperatures above 5000K.In the vaporous state the element, depending on the temperature will either absorb (atomic absorption) or emit (atomic emission) light at very narrow wavelength bands. The wavelength of light absorbed or emitted is characteristic to each element. The wavelength and energy of the light absorbed or emitted is quantified by the

spectrometer. The quantity of absorbed or emitted energy is proportional to the concentration of the element in the sample. A single sample solution containing concentrations of more than 20 elements can be measured with' great sensitivity in less than 1 minute by these techniques.

6.6 NUTRIENTS ROLES AND DEFICIENCY SYMPTOMS

Each mineral element has one or more structural or functional roles in the plant. Inadequate supply of an essential element results in 'nutritional disorder, which is manifested by characteristic deficiency. Such visually observed symptoms include stunted growth of roots, stems or leaves and chlorosis or necrosis of various organs. Characteristic symptoms often help in determining the necessary functions of the element in the plant. Knowledge of nutrient deficient symptoms also helps agriculturists and foresters to determine how and when to fertilize the crops. The deficiency symptoms and the functional roles of mineral elements in plants are described below.

Carbon, hydrogen and oxygen: Although these are not minerals in true sense but as they are very important and necessary for life, therefore, they are included in the list of mineral elements.
Air and soil are the sources from which they are supplied in the form of CO_2 and H_2O.

They are required in the structural backbone of all organic molecules. A deficiency of carbon produces rapid starvation of the plant, while a deficiency of water leads to desiccation.

Nitrogen: Soil is the chief source of nitrogen. Plants absorb this element as inorganic nitrate ion (NO_3^-) or ammonium ion (NH_4^+) from the soil solution. Nitrogen is not a mineral element, but it has been included in the list because it is normally obtained by the plant from soil. Soil gets this element by the activity of atmospheric N_2 fixing bacteria and cyanobacteria.

Nitrogen is a constituent of amino acids, proteins, nucleic acids, chlorophyll and certain hormones and lipids. Thus, processes like protein and chlorophyll synthesis and role of other biomolecules are related to nitrogen nutrition.

Nitrogen deficiency causes slow and stunted growth and chlorosis (yellowing) of leaves. Chlorosis is generally confined to 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 do not show these symptoms initially, because nitrogen can be mobilized from older leaves. Thus, a nitrogen deficient plant may have light green upper leaves and yellow or tan lower leaves (i.e., older). Nitrogen deficiency is also manifested in the form of slender and often woody stems. This woodiness may be due to the accumulation: of excess carbohydrates, that in the absence of nitrogen, cannot be used in the synthesis of amino acids and other nitrogen containing compounds. Also, the carbohydrates that are not used in nitrogen metabolism may be used in synthesizing anthocyanins. This condition is exhibited as purple coloration in stems, petioles and underside of leaves. Further excess nitrogen supply gives ahigh shoot/root ratio and delay in the onset of flowering.

Potassium: Potassium is an essential element for all living organisms. Plants have taken up this element is monovalent cation K+, It is produced in the soil from parent rocks such asfelspar, mica and glauconite by weathering processes.

Potassium does not serve as the component part of any organic compound. Within plants it occurs as the cation K+, It serves as an activator of several enzymes including enzymes involved in photosynthesis and respiration. Potassium also functions in starch and protein synthesis. Further, potassium as an osmoregulatory can control the plant movements such as opening and closure of stomatal guard cells and daily changes in the orientation of leaves (sleep movements).

Like nitrogen, potassium is highly mobile element in plants. Its deficiency symptoms, therefore. First appear in older leaves. The first observable symptom of potassium deficiency is-mottled or marginal chlorosis followed by necrotic lesions (spots of dead tissue) at the leaf tips, margins, and between veins. In many monocots, these necrotic lesions may initially form at the leaf tips and margins and then extend towards the leaf base. The potassium deficient plants may be weak, slender, and shortened stems with increased susceptibility to root-Totting fungi. These effects cause the plant to be easily bent to the ground(lodging).

Phosphorous: Phosphorous in soils occurs mainly in the form of phosphoric acid (H_3PO_4). Plants absorb this element either as monovalent (H_2PO_4) or divalent ($H PO_4^{2-}$) or triphosphate anions. The availability of these phosphorous forms to the plant depends on soil PH. This is because that phosphoric acid contains more than one proton, 'each with a different dissociation constant. At a soil pH less than 7.8, it exists as $H_2PO_4$and between P^H 7.8 and 7.2, it is predominantly HPO_4^{2-}. Soils with pH greater than 7.2 i.e., alkaline soils have phosphorous mainly in the form of trivalent HPO_4^{3-}.This form of phosphorous is not available for uptake by plants. Further, the tendency of phosphorous to form insoluble complexes with aluminum and iron at neutral Pand with calcium and magnesium in alkaline soils, made it a limiting element in soils. Plant roots infected with mycorrhizal fungus have enhanced uptake of phosphorous. Phosphorous is a constituent of many vitally important compounds like sugar-phosphate intermediates of photosynthesis and respiration, phospholipids of cellular membranes. Nucleotides of energy metabolism and DNA and RNA make up. Another organic P containing compound called phytin is present mainly in seeds. Phosphorous in the phytin form of seeds is regarded as a Preserve.

Plants suffering from phosphorous deficiency are retarded in growth. The shoot/root dry matter ratio is usually low. Generally, the symptoms of phosphorus deficiency appear on the older leaves, which are often a darkish green colour. In the extreme situation, the leaves may be malformed and contain small spots of dead tissue called necrotic spots. The 'stems of many annual plant species contain a reddish coloration due to anthocyanin formation in phosphorous deficient conditions.

Sulfur: Plants mainly absorb sulfur in' the form of divalent sulfate anion (SO_4^{2-}). Sulfur oxidizing, soil microorganisms release it to the soil solution from iron sulfides and elemental sulfur. Sulfur is a constituent of amino acids such as cysteine and methionine which contribute disulfide bridges in the tertiary structure of proteins. it is also a constituent of vitamins like thiamine biotin and coenzyme A. Iron-sulfur proteins that contain Fe-S, 2Fe-2S and 4Fe-4S clusters to catalyze electron transfer reactions of photosynthesis, respiration and nitrogen fixation involve sulfur as an integral part. The characteristic odor of Brassicaceae (crucifers) members such as cabbage, onion, garlic and turnips is due to the presence of sulfur as constituent of volatile mustard oils such as thiocyanates and isothiocyanates.

Sulfur deficiency is responsible for chlorosis, stunting of growth and anthocyanin accumulation. Many of these symptoms are like those of nitrogen, because both are

constituents of proteins. However, chlorosis caused by sulfur deficiency appears initially in young leaves rather than on old leaves as in nitrogen deficiency. This is due to its inability to mobilize in most plant species.

Calcium: Calcium in the soil occurs as Ca bearing AI-silicates, Ca phosphates and Ca carbonates such as calcite ($CaCO_3$) or dolomite ($CaCO_3MgCO_3$). The weathering of these Ca bearing primary minerals release Ca2+ cations to be taken up by the plants. Calcium is a constituent of middle lamellae of cell walls. It is also used in the mitotic spindle formation during cell division. In various signal transduction pathways, calcium acts as a second messenger. As a second messenger calcium by binding with a protein calmodulin, forms Ca^{2+-} calmodulin complex in plant cells. This complex regulates the activities of number of enzymes necessary to produce response.

The deficiency of calcium mainly appears in the meristematic regions, where cell division and wall formation arc more rapid. Calcium' is relatively immobile and the symptoms typically appear. First in young tissues such as the tips of roots or young leaves. The young leaves are deformed and necrotic with downward hooking. The root system of a calcium deficient plant may appear brownish, short and give 'slippery' to the touch due to the deterioration of the middle lamella.

Magnesium: Plants taken up magnesium as a divalent Mg^{2+} cation from the soil solution where it is held as an exchangeable base. Magnesium is a constituent of chlorophyll porphyrin ring structure. It is also required to stabilize ribosome structure and to activate enzymes involved in respiration, photosynthesis and the synthesis of DNA and RNA.

A characteristic symptom. of magnesium deficiency is chlorosis due to the breakdown of chlorophyll at interveinal regions. Chlorosis appears first in the older leaves because of the mobility of magnesium. This interveinal pattern of chlorosis due to Mg deficiency results because chlorophyll in the vascular bundles remains unaffected for longer periods than the chloroplasts in the cells between the leaf veins.

Iron: Plant species absorb iron in the form of ferric (Fe^{3+}) or ferrous (Fe^{2+}) ions. The availability of iron to plants increases with increasing acidity of the soil. Iron deficiencies are therefore common in neutral or alkaline soils where the more available form of Fe^{3+} is converted into insoluble hydrousoxides ($Fe_2O_3.3H_2O$). In such cases plants exhibit some specialized iron uptake mechanisms. These include the synthesis and release of iron chelating substances like caffeic acid and phytosiderophores.

Under conditions of iron stress plant roots also exhibit enhanced proton secretion into the soil surrounding the roots. This causes acidification of the soil. Acidification of the rhizosphere then encourages the chelation of the Fe^{3+} with caffeic acid. This complex then moves to the root ‘surface where Fe^{3+} is reduced to Fe^{2+} by the root plasma membrane bound reducing enzyme. This reaction causes iron to enter the root cells and releases chelator to the rhizosphere soil for the next round of iron absorption. The role of plant phytosiderophores in iron uptake processes is a recent discovery. Phytosiderophores are highly specific iron binding ligands found in the members of the family Gramineae. These ligands convert insoluble iron into soluble form by forming a complex called iron phytosiderophore complex or ferrisiderophore complex. The entire ferrisiderophore complex is taken into the root cell, where the iron is subsequently reduced to $Fe^{2+}$and released for use by the cell. Iron is a component of heme containing cytochromes and non-heme iron sulfur proteins. Both these

proteins are important in the oxidation-reduction reactions of photosynthesis and respiration. Iron of these proteins as an electron carrier reversibly oxidized from Fe^{2+} to Fe^{3+}state during electron transfer. Iron is also a constituent of several oxidase enzymes such as catalase and peroxidase. Like magnesium iron deficiency results in intravenous chlorosis. In contrast to magnesium deficiency the symptoms of iron appear initially on the younger leaves, because the mobility of iron in the plant is very low and cannot be withdrawn from the older leaves. Although iron is not a constituent of chlorophyll, the leaves become chlorotic because it is required for the synthesis of some thylakoid electron transport proteins. Under conditions of extreme or prolonged deficiency, the whole leaf becomes white due to impaired protein synthesis.

Boron: is probably taken up by plants as the undissociated boric acid (H_3BO_3). The role of boron in plant nutrition is least understood of all the plant nutrients. The available evidence, however, suggests that it is required for cell elongation, membrane/function, nucleic acid synthesis and hormone responses. In addition, boron is known to' stimulate pollen tube germination and elongation.

Boron deficiency gives the roots stubby and bushy appearance. This is due to inhibition of both cell division and elongation in primary and secondary roots. Inhibition of cell division and elongation in boron deficient plants is accompanied by an increased activity of enzymes that oxidize the hormone indole-3-acetic acid (IAA) and a decrease in RNA content possibly through impaired synthesis of RNA precursor uracil. Other symptoms of born deficiency include shortened internodes resulting in a bushy or rosette appearance. The most well known born deficiency symptoms are stem crack in celery and heart rot in sugar beet.

Copper: Copper is taken up by the plants as a divalent cupric ion, Cu^{2+}. Its availability to plants is influenced by several factors such as soil organic matter, P^H and microorganisms. Copper is a constituent of many oxidative enzymes such as plastocyanin, cytochrome oxidase, amide oxidases, super oxide dismutase and polyphenol oxidase. Most of these copper bound enzymes are involved in oxidation and reduction reactions while some react with O_2 and reduce it.to H_2O_2 or H_2O.

The initial symptom of copper deficiency is the production of dark green leaves, which may. contain necrotic spots. Necrotic spots appear first at the tips of the young leaves and then extend toward the leaf base along the margins. Under extreme copper deficiency, loss of young leaves occurs particularly in fruit trees. This condition is known as summer dieback in citrus trees.

Zinc: Plants use zinc in the form of divalent cation (Zn^{2+}).As a plant nutrient zinc functions as an activator of many enzymes such as alcohol dehydrogenase, carbonic anhydrase, lactate dehydrogenase, glutamic dehydrogenase, alkaline phosphatase and carboxy peptidase. It is also required for the synthesis of tryptophan, a precursor of Indole-3-acetic acid (IAA).

Zinc deficiency is characterized by shortened internodes and smaller leaves, a condition usually referred to as *"little leaf'*. These symptoms may result from loss of the capacity to produce enough IAA. This is because that zinc is required 'for the synthesis of hormone precursor tryptophan.

Manganese: Manganese exists in the soil as a divalent, trivalent and tetravalent forms, but it is absorbed largely as the divalent manganous cation Mn2+.Manganese act as an activator of

several enzymes in plant cells. Enzymes like decarboxylases and dehydrogenases involved in the tricarboxylic acid cycle are specifically activated by manganese. It is an important component of the oxygen evolving complex (OEC) of photosynthesis, where it is in the form of manganoprotein causes the photolysis of water and consequent evolution of oxygen on the lumen side of the thylakoid membranes.

The major symptom of manganese deficiency is intravenous chlorosis with a small necrotic spot on younger or older leaves. Manganese deficiency is also responsible for grey speck of cereals, a disorder characterized by the appearance of greenish-grey oval-shaped spots on the basal regions of young leaves.

Molybdenum: Plants absorb molybdenum in the form of molybdate (MoO_4^{2-}) ions. Molybdenum is an essential component of nitrate reductase and nitrogenase. Nitrate reductase catalyzes the reduction of nitrate to nitrite for its assimilation into amino acids and nitrogenase, an enzyme of nitrogen fixing organisms converts atmospheric nitrogen to ammonia.

Molybdenum deficiency is indicated by chlorosis and necrosis between veins of the older leaves. Plants that can depend on nitrate or symbiotic nitrogen as source of nitrogen are usually subjected to molybdenum deficiency. In such plants *whiptail*is a common disorder in which young leaves are twisted and deformed. Premature flower fall or prevention of flower formation are other molybdenum deficiency symptoms.

Nickel: Nickel has only recently been added to the list of essential elements. Plants absorb it in the form of monovalent Ni^+ cation.

Nickel is an integral part of two enzymes namely urease and hydrogenase. Urease catalyzes the hydrolysis of urea into NH_3 and CO_2, whereas hydrogenase in nitrogen fixing bacteria catalysis the recycling of hydrogen gas generated during N_2 fixation.

Nickel deficiency in plants is very rare, because they require this element at very low quantities. A nickel deficient plant accumulates urea in its leaves. Such leaves show leaf tip necrosis.

Chlorine: It occurs commonly in soils as chloride anions (CI^-) and move freely in soil solution from which it is available to plants. Most plants absorb chlorine at levels much higher than is needed for normal functioning. Along with manganese, chloride is required for water splitting reactions of photosynthesis. It is a counter ion in the maintenance of electrical neutrality across the energy transducing membranes.

Like potassium, it is one of the osmotically active solutes in the vacuole. Chloride also required for cell division in both leaves and roots.

Plants deprived of chloride tend to exhibit reduced growth, wilting of the leaf tips and a general chlorosis. Roots of chlorine deficient plants may appear stunted and thickened near the root tips.

Other nutrients: In addition to the 17 essential elements described, for some plants there is a requirement of some additional elements such as sodium, silicon, cobalt and selenium. Plants

require these elements at quantities that cannot be detected reliably through the presently available analytical techniques. Hence, they are called beneficial elements.
Sodium is required as a 'micronutrient' for most of the plant species that have C4, and CAM pathways of carbon fixation. In these plants, sodium ions (Na+) are required for regenerating phosphoenol pyruvate from pyruvic acid. Phosphoenolpyruvic acid is the first CO_2 accepter in C4and CAM plants. Sodium deficiency in these plants causes chlorosis and necrosis.

Silicon is a beneficial element particularly to grasses. In these plants it is present as a constituent of cell walls of epidermal cells. Its deficiency leads to lodging of these plants.

Cobalt is necessary for the growth of both symbiotic and asymbiotic nitrogen fixing microorganisms. In the absence of this beneficial element, nitrogen nutrition of legumes may. be affected.

Generally, **selenium** is toxic to most plant species. However, certain plant species called seleniferous indicators such as *Astragalus bisulcatus*and *A_pectinatus*accumulate high selenium that would be toxic to most other plants. The exact role of selenium in these plants is not yet known.

Plants containing high selenium content exhibit sickness known as alkali poisoning. Plants require every essential mineral element at a particular concentration. The concentration of the nutrient at which plants show maximum growth or yield is defined as **critical** concentration

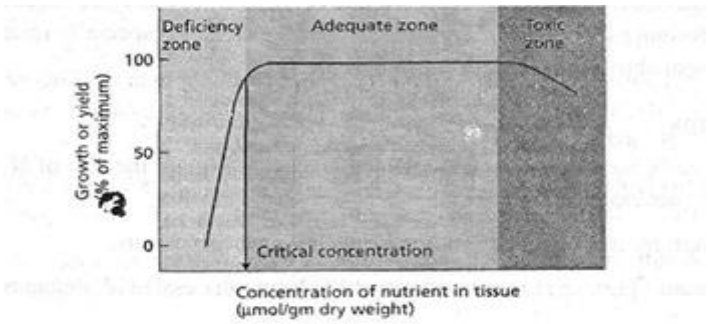


Figure 6.4 Relationship between growth and the nutrient content of the plant tissue,

(Figure 6.4). As the nutrient level in the tissue sample increases above critical concentration, a point is reached at which additional increases of mineral nutrient have no effect on growth or yield. Such a nutrient concentration is called **adequate concentration.** When the nutrient content in a tissue sample is low, growth is reduced. At this point the nutrient level is said to be deficient. As the nutrient content of the tissue increases above the adequate level, growth or yield declines because of toxicity. For example, the critical concentration of copper in plants ranges from 4 to 15 µg g-1 of tissue dry weight. The growth is reduced when it *is* present below 4 µg and becomes toxic more than 20 µg.

6.7 SUMMARY

Plants are autotrophic organisms. They require mineral elements to be used in biosynthesis and energy production. Studies of plant nutrition have shown that plants require seventeen elements obtained from the soil. These 17 elements are considered essential because it has been demonstrated that in their absence all plants are unable to complete a normal life cycle.

The seventeen essential elements are classified as macro nutrients and micronutrients, depending on the relative amounts required.

Macronutrients (C, 0, H; N, P, K, S, Ca and Mg) are needed in large quantities and micronutrients (Fe, B, Cu, Zn, Mn, Mo, Ni and CI) are used in very small amounts. Techniques such as ash analysis, solution culture and atomic absorption or atomic emission spectrophotometer is used in the study of mineral elements essential for plant life, Each essential mineral element has a role to play in the biochemistry and physiology of the plant. A plant that is deficient either in macronutrient or micronutrients exhibits a nutritional disorder with characteristic symptoms. Nutritional disorders occur because nutrients serve as components of organic compounds, in energy storage, to maintain plant structures, as enzyme cofactors, and in electron transfer reactions. - .

There are some additional nutrients called beneficial elements which may be required by some plants to satisfy some special requirements. Essential elements, especially micronutrients,may be toxic when present above the critical concentration.

6.8 MODEL QUESTIONS

1. What are the criteria for essentiality of mineral elements? Explain the role of N P and K inplant growth and development.
2. Discuss the symptoms and effects of micronutrient deficiency in plants.
3. What do you mean by mineral nutrition in plants? Name the essential elements with theirroles.
4. Write short notes on :
   (a) Iron as plant nutrient
   (b) Sulfur and calcium deficiency
   (c) Solution culture

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Dr. Madhuri vajha

LESSON -7

STRUCTURE, FUNCTION AND MECHANISM OF PHYTOCHROME ACTION AND IT ROLE IN GENE EXPRESSION, AND CRYPTOCHROME

OBJECTIVES :

In this lesson, you will learn:

- The concept of light-regulated plant development or photomorphogenesis.
- The discovery of phytochrome and review the basic chemistry of this' uniquely photo reversible pigment.
- The physiological effects of phytochrome, showinghow.it is. involved in every aspect of development.
- How phytochrome can be used to monitor changes in the natural light environment.
- How phytochrome works at the molecular level.
- Responses of plants to blue light and UV -B radiation.

CONTENTS

7.1 INTRODUCTION

Everybody is familiar with the fact that the life of a green plant depends on light. During photosynthesis, light energy is converted into organic matter. The first scientific documents of photomorphogenesis was described by Julius van Sachs, the founder of modern experimental plant physiology (Fig. 7.1). Sachs noted that darkened seedlings or parts of older plants developed an irregular, misshapen appearance, characterized by a thin, elongated stem and rudimentary, yellow leaves. He described this syndrome as an "etiolation illness" which could never be cured but be alleviated, if another part of the plant was exposed to light.

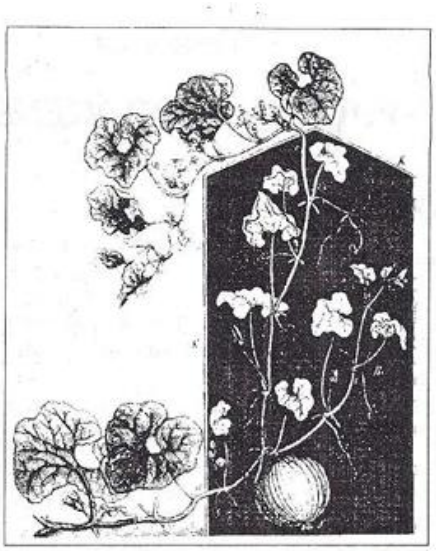

Fig. 7.1 Experimental arrangements used by Julius von Sachs to demonstrate the etiolation syndrome of a partly darkened plant.

According to today's meaning, 'Photomorphogenesis' embraces all regulatory effects of light (visible and near-ultraviolet parts (300-800 nm) on the development of plants, independent of photosynthesis. The developmental effect of complete darkness is designated as 'skotomorphogenesis' or 'etiolation'.

Photomorphogenetic processes utilize radiant energy to "trigger" or initiate reactions that control or alter growth, development or differentiation photomorphogenetic reactions are initiated by low levels of radiant energy. In many plants, for example, photosynthesis may be driven by solar flux densities in the range of 1000 to 1200 Wm-2 , whereas photomorphogenetic reaction, such as seed germination, is triggered at flux densities of around 0.01 to 0.1 Wm-2. Moreover, in photosynthesis radiant energy must be supplied continuously, whereas in photomorphogenesis a brief exposure to an appropriate radiation source may suffice to set the process in motion.

The photoreceptors used in photomorphogenesis differ from the photoreceptors of photosynthesis. Chlorophyll, phycobilin or rhodopsin pigments are present in large amounts in the cell and are densely packed in membranes specialized to convert light energy into chemical energy. In contrast, photomorphogenetic photoreceptors must be conceived as sensory pigments. The cellular level of these is extremely low and the function is not dependent on membranes.

Responses to red light: For many years, it was known that plants respond to low levels of irradiation by several different growth responses, such as etiolation and bending. It was observed that the seeds of some plant species did not germinate if maintained in the dark in a fully imbibed condition. If such seeds were given a brief exposure to light, germination proceeded in a normal manner.

In the 1930s, two scientists L.H. Flint of the U.S. Department of Agriculture & E.D. Me Alister of the Smithsonian Institution, were studying the germination of light-sensitive seeds under light or different wavelengths obtained from a series of filters. They reported in 1935

that the seeds of Lettuce *(Lactuca sativa* cv. Grand Rapids) were promoted to germinate by light in the spectral region of 525 to 700 nm. The optimal promotive effect was noted around 660 nm, high referred to as red light. They also found that, far-red light, i.e. 700 to 820 nm, has no promotive influence on the germination of lettuce seeds.

Dry seeds of Grand Rapids lettuce do not germinate, nor dry seeds respond to light. Seeds were allowed to imbibe water from water-soaked blotters in petri dishes for 16 hours in complete darkness. If left in darkness for a further 32 hours, a few seeds germinate. All the seeds germinated when 16 hour dark imbibed seeds were exposed to a brief flash of white light or sunlight and returned to darkness for an additional 32 hours. If the dark period was interrupted at the end of 16 hours with varying wavelengths rather than white light or sunlight, seed germination was promoted by red light (660 nm). Far-red light did not promote germination. Seed germination was inhibited, if seeds promoted to germinate by red light were given an immediate exposure to far-red light (730 nm). The promotion of seed germination in red light (660-680 nm) and the inhibition of seed germination in far-red light are reversible.

Most photomorphogenic responses in higher plants appear to be under control of one of three signal transducting photoreceptors:

(1) phytochrome, which absorbs in the red (R) and far-red (FR) regions of the spectrum.
(2) a blue and UV-A-absorbing receptors, cryptochrome.
(3) one or more UV-B-receptors.

7.2 PHYTOCHROME:

It is now well established that the ubiquitous chromo-protein called phytochrome plays a critical role in almost every stage of plant development. Its existence was predicted based on a simple physiological observation, seed germination and growth of etiolated seedlings exhibited photo reversible responses to red and far-red light. In 1950s, H.A. Borthwick, a botanist, and S.B. Hendricks, a physical chemist, and their colleagues began a study of action spectra for such a phenomenon as germination of photosensitive lettuce seeds, pea stem elongation and photoperiodic control of flowering. One exciting observation was the similarity of action spectra, with peaks in the red and far-red. This was the discovery of photoreversibility - a response potentiated by red light could be negated if the red light treatment were followed immediately with far-red light. 'In 1960, they proposed that the seeds contain a pigment and named the pigment as 'Phytochrome:

This pigment exists in two forms: a red absorbing form called Pr and a far red-absorbing form called Pfr(Fig. 7.2)

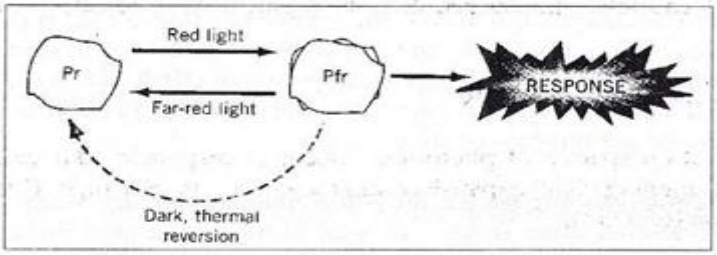

Fig. 7.2 The photoreversible pigment system

In an experiment, groups of seeds were imbibed with water in darkness for three hours before
'
subjected to light treatments. The light treatments were either 1 minute' of red light or 4 minute of far-red light. Following irradiation, the seeds were returned to darkness for 48 hours. The number of germinated seeds in each lot were counted (Table 7.1).
Table 7.1 Photoreversible control of germination

Irradiation	Germination (%)
R	88
R, Fr	22
F, Fr, R	84
F, Fr, R, Fr	18
R, Fr, R, Fr, R	72
R, Fr, R, Fr, R, Fr	22

When the Rand FR treatments are alternated, the % germination appears to depend on whether R or FR was presented last. –

Beltsville group has predicted several features of this hypothetical pigment system.

- because seeds and dark grown seedlings tissues responded initially to red light, the pigment was probably synthesized as the Pr form, which accumulated in darkness. Pr was stable and probably physiologically inactive.
- because treatment with red light initiated germination and other developmental events Pfr was probably the active form. Pfr was unstable and was either destroyed or could revert to Pr in darkness by a non-photochemical, temperature-dependent reaction.
- because the pigment could not be seen in dark-grown', chlorophyll-free tissue,it was at a very low concentration.

Borthwick and Hendricks summarized that, the pigment must be acting catalytically and was a protein.

7.2.1 Structure and Properties of Phytochrome

Chromophore: The action spectra of photomorphogenetic responses like seed germination, stem elongation, hook opening, leaf expansion, anthocyanin and chlorophyll synthesis show maxima in the red region (660 nm).

The chemical structure of phytochrome (Pr form) the tetrapyrrole chromophore is covalently linked to apoprotein via a thioester bond (-8-) to the vinyl group of ring A. The chromophore-binding crevice is hydrophobic. Additional hydrophobic site on protein moiety binds flavor (Fig. 7.3).

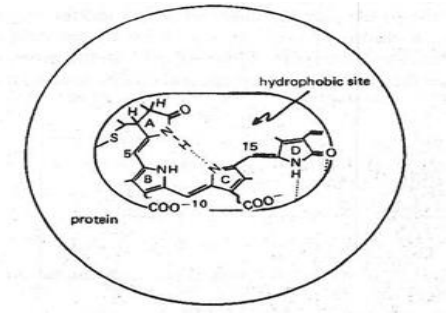

Fig. 7.3 Covalent linkage of chromophore to apoprotein

The cyclic Pr-chromopeptide can be photo-isomerized to a semi-extended chromopeptide in the presence of thiol, acts as nucleophilic catalyst.

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The Pfr chromophore results from the photo-induced addition of amino acid residue to the ring A methene bridge (Fig. 7.4)

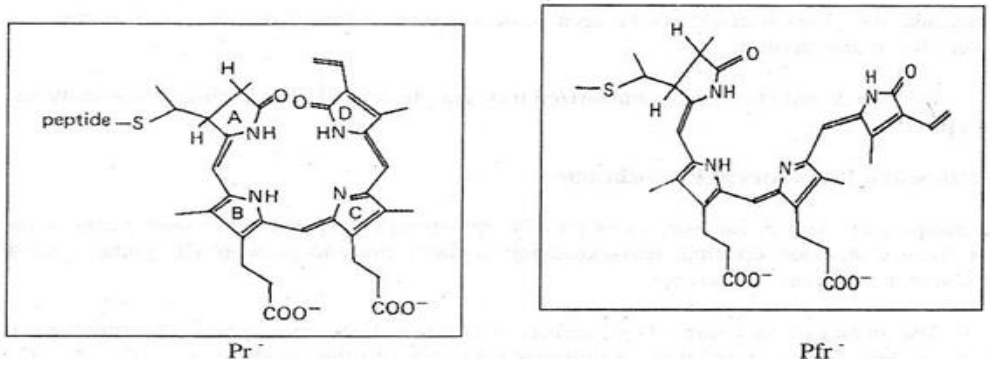

Fig. 7.4 The chromophore structures of Pr - and Pfr – chromopeptide

Spectroscopy: The absorption spectra of phytochrome are qualitatively like those of 'porphyrins and chlorophylls with their characteristic visible and Soret bands. Note differential absorption in the blue region of the 'spectrum as well as the red/far-red region. Some blue light effects are mediated by phytochrome, but photo conversion by red light is 50 to 100 times more effective than the blue. Because both forms absorb equally in the green region (500 to 550 nm), green light does not change the state of the pigment and can be used as a safe light (Fig. 7.5).

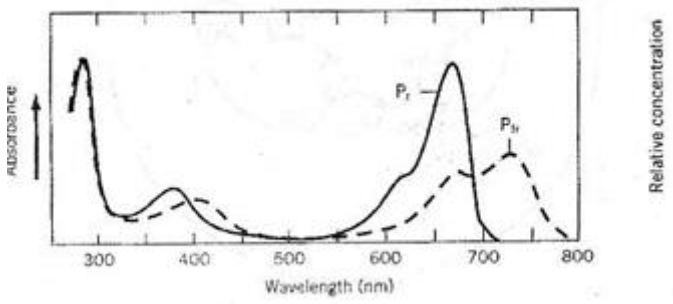

Fig. 7.5 Absorption spectra of purified phytochrome

Phytochrome is believed to exist *in vivo* as a dimer with one chromophore per monomer. **It** appears that crude extracts of all plants contain a Pr-specific protease that cleaves the protein into several fragments. Isolation of intact, or native, protein can be optimized by first converting the pigment to the Pfr form, adding protease inhibitors and working rapidly at ice temperature. Molecular mass estimates for native monomers range from 120 kDa to 127 kDa (maize).

The molecular mass of oat phytochrome is 124 kDa. A polypeptide map containing 1128 amino acids has been deduced from DNA nucleotide sequence analysis. The chromophore is attached at cysteine-321, part of a unique l l-amino acid sequence at the NH2 terminal end of the protein (Fig. 7.6). Chemical data indicates that the chromophore is housed within a cavity in the folded protein, shields the chromophore from the external aqueous environment.

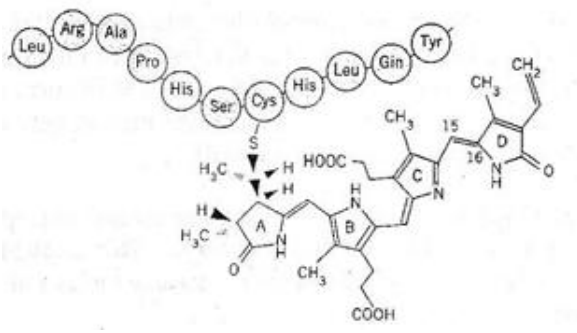

Fig. 7.6 Structure of the phytochrome chromophore and its binding to the apoprotein.

As noted earlier Pr is biologically inactive and that formation of Pfr initiates an active physiological response. The exact nature of the phototransformation between the two forms is not clear, though, both the chromophore and the apoprotein are believed to' undergo confirmational changes (Fig. 7.7).

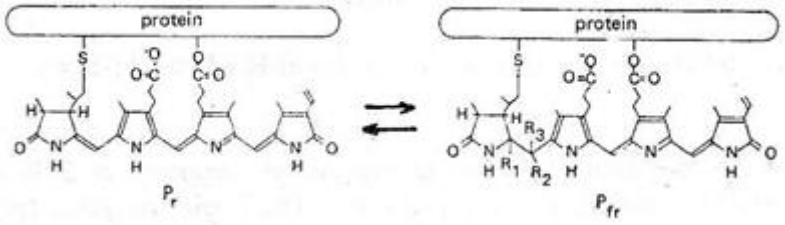

Fig. 7.7 A mechanism of the phototransformation of phytochrome based on the addition of the 4-5 double bond.

The principal difference between the Pr chromophore and the Pfr chromophore appears to be a *cis-trans* isomerization of the methine bridge between rings C and D. The absorption of red light provides the energy required to. overcome a high activation energy far rotation around the double bond, which is not normally achieved at ambient temperature.

Protein moiety

Siegelman & Firer (1964) reported a molecular weight of 90,000-1,50~000 for rye phytochrome. Many different values for the molecular weight of phytochrome have appeared in the literature. Three factors seem to have contributed to this confusion:

(1) different plant sources,
(2) proteolytic degradation during isolation,
(3) the tendency of phytochrome to aggregate.

It appears that intact / undegraded phytochrome has a molecular weight of 1,24,000 per monomer. A 6000~dalton peptide fragment is readily lost from intact phytochrome, when the Phytochrome is in the Pr form. Thus, the susceptibility of the Pr form to proteolysis accounts for the earliest observation that phytochrome isolated in the Pfr form exhibits a higher molecular weight than that isolated in the Pr form upon SDS PAGE.

The most significant spectral difference between intact and large phytochromes is the red shift of the visible absorption maximum in the Pfr form. This explains why the absorbance

maximum at > 730 nm for Pfr *in vivo* or crude extracts obtained after red irradiation of tissues is longer than that obtained for Pfr *in vitro.*

Amino acid composition and sequence

Immuno affinity - purified large oat phytochrome of greater than 98% purity that exists in solution as a dimer of its 118,000 molecular weight monomers contains about 35% non-polar amino acid residues, with 115 carboxylic amino acids per monomer (Table 7.2). The following phytochrome sequence has recently been elucidated.

Leu-Arg-Ala-Pro-His-Ser-Cys (-S-Chromophore)-His-Leu-Gln- Tyr

Protein structure: The large phytochrome structure is composed of 20% a-helix, 30% β . pleated sheet and 50% random coil confirmations. The isoelectric point (pI) on the surface charges large phytochrome ranges from 5.8 to 7.G depending on the source and proteolytic modification of *in vitro.*

Table 7.2 Amino acid compositions of large phytochrome expressed as residues(rounded to the nearest integer) per - 120,000- dalton subunit.

Amino acid	Rye phytochrome[a]	Oat phytochrome[b]	Oat phytochrome[c]
Lys	58	64	63
His	28	34	33
Arg	47	51	50
Asp	104	118	112
Thr	46	38	44
Ser	75	73	80
Glu	128	122	122
Pro	88	45	44
Gly	77	72	77
Ala	110	93	93
Cys	26	27	16
Val	89	79	81
Met	32	26	31
Ile	54	51	52
Leu	111	119	114
Tyr	23	23	21
Phe	43	45	43
Trp	--	8	9
Total residues	**1139**	**1088**	**1085**

[a]Data from Rice and Briggs (1973).
[b]Data from Hunt and Pratt (1980).
[c]Data from Roux *et al.* (1982).

7.2.2 Phytochromes in green plants

Phytochrome in green plants is different from phytochrome in etiolated. tissues. The form of phytochrome expressed in etiolated tissues is only one of five gene products. Four other gene products are expressed at low levels in both dark and light grown tissues. If Pfr is degraded as rapidly in green plants as it is in etiolated tissues, how do plants under continuous illumination maintain adequate levels of phytochrome? It has long been argued (based on both physiological and *in vivo* spectrophotometric studies) that some properties of phytochrome in light-grown green plants differed from those of etiolated seedlings.

Phytochrome from light-grown *Avena* tissue is smaller (118 kDa) and has a shorter Pr absorption maximum (652 nm) compared with the pigment from etiolated tissue (124 kDa; 666 nm). Phytochrome is neither immunoprecipitated nor recognized on immunoblots by antibodies raised against phytochrome from etiolated seedlings. The kinetics of photoconversion (Pr to Pfr and back to Pr) appear to be similar, but Pfr in light-grown tissue has a longer half-life. The half life of Pfr in treated seedlings grown under continuous light is about 8 hours compared with 1.0
to 1.5 hours in etiolated seedlings.

The labile form of phytochrome that accumulates in dark-grown seedlings is called as Type I phytochrome, the more stable form found in green seedlings is called Type II phytochrome. Recent studies using recombinant DNA techniques have shown there are multiple forms of Type II phytochrome, encoded by a small family of differentially regulated genes.

The best characterized family of genes has been isolated from *Arabidopsis thaliana*. There are five phytochrome genes, *phyA, phyB, piiy C, pnyt»* and pf1yE. The gene phyA is expressed in dark-grown tissue. This encodes the Labile Type I form of phytochrome (PHY A). This accumulates in dark. Transcription of phyA is inhibited by PfrA (far-red absorbing form of PHY A), so PHY A does not accumulate in the light. PHY A protein is rapidly degraded. The remaining Type II phytochrome genes (phyB, phyC, phyD & phyE) are expressed at low levels in both light and darkness. Their products (PHY B-E) are light stable.

Finally, the accepted dogma of phytochrome is that Pr is biologically inactive and formation of Pfr initiates active developmental responses. Recently, it has been shown that the normal vertical growth habit of Arabidopsis seedlings is reduced when grown in red light. On the other hand, mutants that lack photochemically functional phytochrome and are unable to produce Pfr, exhibit a normally erect habit regardless of light treatment. Liscum and Hangarter have concluded that the normal erect growth habit occurs when phytochrome B is in the Pr form. It is assumed that the erect habit is the "active response".

7.2.3 Physiological effects of phytochrome

Phytochrome mediated effects are conveniently grouped into three categories based on their energy requirements. The classical red, far-red photo reversible responses discovered by Hendricks & Borthwick are known as low fluence responses (LFRs). Photonfluence requirements for LRFs are in the range of 10^1 to 10^2 umol m^{-2} of red light. Very fluence responses (VLFRs) are induced by much lower light levels, 10^{-6} to 10^{-3} umol m^{-2} red light. High irradiance reactions (H1Rs) require continuous irradiation. Photoinhibition of seed germination appears to be an example of a high irradiance reaction (Fig. 7.8).

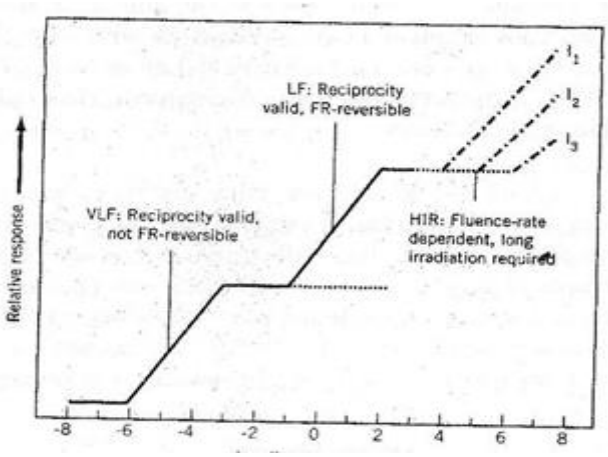

Fig. 7.8 The three categories of Phytochrome

1) Low Fluence Phytochrome Responses

Seed Germination: The germination of most seeds is influenced by light. This includes most non-agricultural species are known as positively photoblastic and germination inhibited by light are negatively photoblastic.

Soil attenuates light very quickly. A 1 mm thickness of fine soil passes less than 1% of the light at wavelengths longer than 70 nm. So most light-requiring seeds need not be buried very deeply for germination. Some seeds eg: *Sinapsis arvensis* require very little Pfr to stimulate germination and may exhibit germination when covered with upto 8 mm of soil. Suppression of germination in negatively photoblastic seeds like oars (*Avena fatua*) requires long-term exposures at high fluence rates.

Seedling development: Plants grown in darkness take an unusual appearance. Generally, the stems of dicot seedlings are very long and spindly with a pronounced recurve just below the leaves. The leaves undergo limited development and remain small and elapsing, as though they were in the embryo. Chlorophyll is absent and the seedlings appear white or yellow in colour.

In monocots like grass seedlings continue to elongate and remain tightly rolled up. The first internode or mesocotyl of grass seedlings elongate excessively in the dark and the coleoptile grows (which is a modified leaf) slowly arrested chloroplast development and low activities of enzymes. This general condition exhibited by dark-grown plants is called **etiolation**.

In dicotyledonous seedlings,' hypocotyl elongation, plumular hook-opening and leaf expansion have received the most attention. Upon irradiation with white light, the growth rate of the hypocotyl slows; the hypocotyl hook straightens, and elongation of epicotyl accelerates, Light stimulates the leaves to unfold-complete leaf development, 'chloroplast development and this proceeds the accumulation of chlorophyll.

A seed carries a limited amount of nutritive tissue that must be sufficient to support the development of the seedling until the seedling is established in the light and photosynthesis can take over the supply of energy and carbon. In the dark, the limited reserves of seed are helped to extend the plumule, composed of young leaves will reach the light and be able to carry out photosynthesis before the reserves are exhausted. Once established, in the light, the remaining reserves may be invested in development of chloroplast, leaf expansion etc. So, the

role of phytochrome in seedling development appears to be one of conveying information, to the seedlings.

In addition to morphological changes in etiolated seedlings, other changes at morphological, biochemical and biophysical are also modulated by phytochrome (Table 7.3).

Table 7.3 Selected example of phytochrome-mediated responses

Photoperiodic floral induction
Nyctinastic leaf movements
Phototropic sensitivity
Seed germination
Stem elongation
Plumular hook opening
Leaf and cotyledon expansion
Chloroplast development
Chlorophyll and carotenoid synthesis
Anthocyanin synthesis
Enzyme activation
Protein synthesis
mRNA transcription
Chloroplast phototactic movement
Surface potential (root tips)
Transmembrane potential

In most LFR experiments, the level of the response with FR, either alone or in sequence with R, is typically higher than dark controls. FR never establishes complete photo reversibility.

Bioelectric Potential and Inn Distribution: The response time for most phytochrome mediated developmental effects are measured in hours or even 'days. But there are some responses which are measured in minutes or seconds. Most of these responses appear to relate to membrane-based activities like bioelectric potential or ion-flux. T. Tanada observed that dark-grown barley root lip would float freely in a glass beaker with a specially prepared negatively charged surface. Within 30 minutes following a brief red irradiation to the root tips would adhere to the surface. A subsequent far-red treatment would release the root tips from the glass. Adhesion and release were correlated with phytochrome-induced changes in the surface potential of the root tips. A brief red treatment generated a positive surface potential, attracting the tips to the negatively charged surface. A far-red treatment generated a negative surface potential, causing the tips to detach.

Phytochrome modulated transmembrane potential has been reported, but in most cases red light induces a depolarization of the membrane within 5 to 10 seconds following a red light treatment. A subsequent far-red treatment causes a slow return to normal polarity or small hyperpolarization.

One of the oldest and most detailed studies of membrane-based phytochrome effects is chloroplast rotation in *Mougeotia,* a filamentous green alga. It contains a single flat chloroplast that can rotate around its long axis so that either its face or its edge is oriented toward incident light (Fig. 7.9).

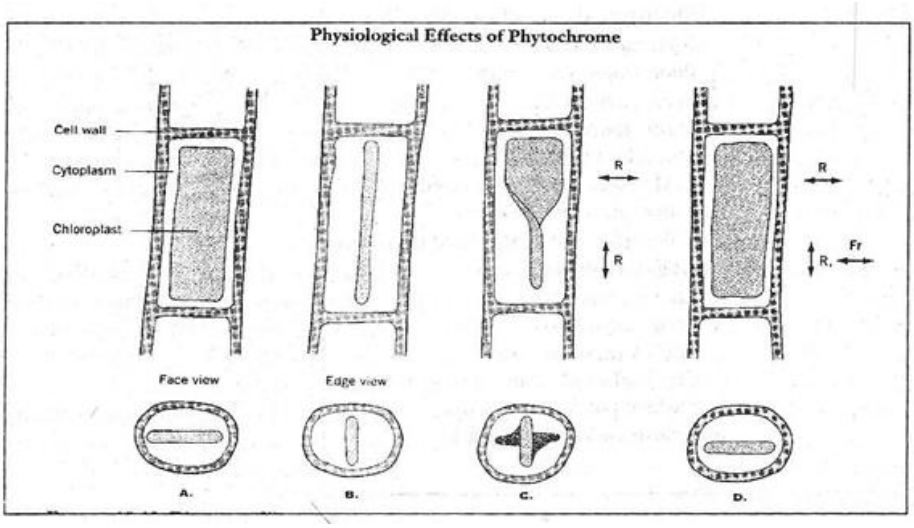

Fig. 7.9 Diagram of Haupt's experiments with *Mougeotia* chloroplasts

Reorientation of the chloroplast is mediated by phytochrome: Red light is most effective, and it is far-red reversible. W. Haupt employed plane-polarized light and microbeams of red and far-red light to irradiate specific locations in the cell. He found that the phytochrome responsible for chloroplast rotation was located not in the chloroplast itself but in the cortical cytoplasm, that is, the region 'of cytoplasm lying inside the plasma membrane. Reorientation of the chloroplast in plane-polarized light was dependent on the direction of polarization relative to the long axis of the cell. Polarized light was most effective when its electrical vector was parallel to the long axis of the cell. Far-red was most effective at reversing the red effect when polarized in a plane at right angles to the plane of red light. These results suggest that the phytochrome molecule assumes a particular orientation in the cytoplasm and that the orientation of Pfr is normal to the orientation of Pr.

There must be a signal chain that links the phytochrome to the chloroplast. Calcium may function as a second messenger for phytochrome, perhaps interacting with the cytoskeleton to control chloroplast orientation. The uptake of Ca^{2+} into Mo*ug*e*oti*a cells is stimulated by red light. Application of the calcium ionophore A23187 to specific sites on the cell wall stimulate the chloroplast to reorient, if calcium is available in the suspension medium.

A correlation between phytochrome and ion movements has been demonstrated in *nyctin*ast*i*c or sleep movements of leaves. Paired leaves or leaflets are generally horizontal during the day but fold together when darkened. Plants that show this behavior have a bulbous zone called the p*ulvinu*s at the base of the leaf or leaflet. The pulvinus drives leaf movement by altering its shape by changing the volume of cells on the upper and lower side of the organ, Changes in the volume and shape of these cells by rapid distribution of solutes like K+, Cl^- and malate. K+ moves through electrically gated K+ channels which are opened by phytochrome-driven H+ efflux. The role of phytochrome may be activate a plasma membrane bound to the ATPase proton pump that inturn depolarizes the membrane to open the K+ channels. Indeed, Ca+2/calmodulin has been implicated as a mediator of several phytochrome responses. It remains to be demonstrated that phytochrome promotes an increase of free Ca+2 in the cytoplasm of plant cells.

Very Low Fluence Responses (VLFRs): A low far-red fluence promotes phototrophic sensitivity as red light does. This indicates that less than 1% of the pigment needs by converted to Pfr in order to saturate the response. D.F. Mandoli and W.R. Briggs (1981)

found that as little as 0.01% Pfr is required to elicit inhibition of mesocotyl elongation. This extreme sensitivity to light makes the study of VLFRs technically difficult. VLFRs are not photoreversible. The evidence that a VLF response is mediated by phytochrome is similar of its action spectrum to the absorption spectrum of PL

High Irradiance Reactions (HIRs)

In the natural environment, plants are exposed to long periods of sunlight at relatively high fluence rates. Under such conditions, the photomorphogenic program achieves maximum expression and responses like leaf expansion and stem elongation. Such light-dependent responses are known as high irradiance responses (HIRs). HIRs show the following characteristics:

(1) full expression of the response requires prolonged exposure to high irradiance,
(2) the magnitude of the response is a function of the fluence rate and duration,
(3) HIRs are not fully red, far-red photoreversible.

Like other responses of etiolated seedlings, the initiation of anthocyanin accumulation is a classic phytochrome-dependent LFR. The red, far-red photorevesibility is limited to brief irradiation when long-term irradiation is applied, the action peak for anthocyanin accumulation is shifted to the far-red, with reduced effectiveness in the red.

Although the effectiveness of red and far-red light argues in favour of phytochrome as a photoreceptor, the unique characteristic of phytochrome reactions, photoreversibility is conspicuously absent from high irradiance reactions.

Based on variations in action spectra, at least three categories of HIRs can be recognized:

(a) action in the blue-UV -A, red and far-red.
(b) action in the blue-UV -A, red and far-red.
(c) action in the blue-UV-A, red and far-red.

To explain these differences, at least two photoreceptors must be involved. Phytochrome and a blue-UV-A receptor.

Hartmann presented seedlings with light of either 658 nm or 766 nm. These wave lengths were chosen because they are absorbed by Pr and Pfr. When presented separately, 658 and 766 nm light were ineffective at inhibiting hypocotyl elongation. When the wavelengths are presented simultaneously both could inhibit elongation as efficiently as 716 nm light. 766 nm light is (Hartman's thesis)' ineffective because it converts phytochrome predominantly I to the inactive Pr form. 658 nm light converts the pigment to the Pfr form that is rapidly lost by degradation reactions. Light at 716 nm is effective because it establishes an intermediate level of Pfr, balancing the competing reaction of Pfr action and Pfr degradation.

In some systems, a separate blue-UV-A receptor interacts with phytochrome in controlling the high irradiance reaction. A blue light-dependent inhibition of hypocotyl elongation in light-grown. seedlings (Cucurbita, Lactuca : and *Lycopersicum* can be demonstrated by simultaneous irradiation with blue and white light. This causes inhibition of stem growth compared with controls receiving white light alone. But these conditions do not alter the ratio of Pfr to total phytochrome.

Finally, phytochrome does not induce anthocyanin biosynthesis in totally dark grown seedlings. Red, far-red photoreversible control follows a prolonged blue light treatment.

These results strongly support that a separate blue-UV -A photoreceptor may be operative in some HIRs and that it may act cooperatively with phytochrome.

7.2.4 Mechanism of phytochrome action

To answer the question, whether the phytochrome molecule is associated with membranes in the cell, there are two strategies for answering this question. The first strategy to be attempted was to fractionate cells and using dual-wavelength difference spectroscopy, assay for phytochrome in the various fractions. Using this approach, phytochrome has been reported in association with every fraction of the cell, including plastids, mitochondria, Endoplasmic reticulum and Plasma membrane as well as the soluble fraction.

The subcellular distribution of phytochrome has also been studied by immunocytochemistry. Material fixed for examination by either light or electron microscopy is probed with antibodies to phytochrome. The antibodies are labelled with enzyme peroxidase or some other marker to make direct visualization of the Ag-Ab complex. In dark-grown coleoptile parenchyma cells, phytochrome appears to be uniformly distributed throughout the cytosol, some of the pigment is associated with the plasma membrane, ER and the nuclear envelope.

Since isolated organelles have phytochrome associated with them, it should be a simple matter to test purified organelles for R/Fr photoreversible functions. Isolated mitochondria have been thoroughly documented and exhibit photoreversible NADP reduction calcium fluxes and ATPase activity.
The evidence indicates that phytochrome is not an intrinsic membrane protein. It may induce changes in membrane properties, by a loose association with the membrane or through an intermediate: but yet unidentified signal chain.

7.2.5 Phytochrome and gene action:

In all physiological events there is participation of phytochrome, which suggests an involvement of gene expression. This gives rise to the expectation that changes in the level of specific gene products i.e. proteins and ultimately mRNA levels are subject to regulation by phytochrome.

Phytochrome regulation at the level of protein was first reported in 1960 by A. Marcus. He reported red far-red reversible control of glyceraldehyde-3 -phosphate dehydrogenase activity in bean seedlings. Nine years later, M. Jaffe reported phytochrome-dependent increases in the RNA content of pea 'buds 24 hours after a red light treatment. In 1985, E.M. Tobin and J.Silverthorne were able to list nine. identified and multiple unidentified proteins whose genes were expressed differently in light-grown and dark-grown plants. Seven of these genes have been shown to be regulated by phytochrome.

Most phytochrome-regulated genes studied so far are nuclear genes encoding, from mRNAs of chloroplast proteins. Two have been studying extensively. The small subunit of' ribulose-I,5-bisphosphate carboxylase/oxygenase (Rubisco) and the light-harvesting chlorophyll a/b binding proteins (LHCP).

Nuclear runoff experiments have confirmed that phytochrome regulates the genes for, both proteins at the transcriptional leve1. In these experiments, nuclei isolated immediately following the light treatment are incubated in the presence of a radiolabeled RNA precursor (^{32}P uridine-triphosphate). Conditions are chosen such that only transcripts that were initiated prior to isolation of the nuclei will incorporate the label. The amount of RNA transcribed from a particular gene can then be measured by hybridizing the labelled transcripts to cDNA known to contain that gene. Results of these experiments confirm that Pfr acts to increase the rate. of transcription of these two genes.

There are two examples of important genes whose transcription is negatively regulated by phytochrome. One negatively regulated gene encodes for NADPH~protochlorophyrlide oxidoreductase. This enzyme catalyzes reduction of protochlorophyllide to chlorophyllide. The level of translatable mRNA decreases within an hour following a brief red pulse and remains low in continuous light. The effect of a red pulse is reversible with far-red. A decrease in mRNA would decrease in activity in light.

The second example of negatively regulated transcription is the phytochrome gene itself 5 seconds of red light causes a rapid decline in translatable phytochrome mRNA in etiolated seedlings. After a 15-minutes lag period, the level of mRNA drops by 50% within the first hour and by more than 95% in two hours (Fig. 7. 10A). The decline in mRNA is far-red reversible though the level of Pfr established by far-red light alone is sufficient to induce loss of mRNA, It appears that phytochrome autoregulates transcription of its own mRNA by some form of feedback inhibition (Fig. 7.l0B).

Phytochrome controls. gene expression and that it can 'do so at the transcription level. Some studies have suggested that Pfr may activate another protein (a second messenger) that binds to certain DNA sequences called light-regulated elements (LREs) (Fig. 7.10A). Binding of the regulatory protein to the LRE stimulates transcription of the gene. In absence of this, protein transcription will not occur. LREs have been identified for two genes, one encodes the small sub-unit of Rubico (rbc S) and the other is the apoprotein of LHCP (cab). The LRE for the rbc S gene will impose light sensitivity on reporter genes that are not normally sensitive to light if the reporter gene is inserted into the chromosome into the region of LRE.

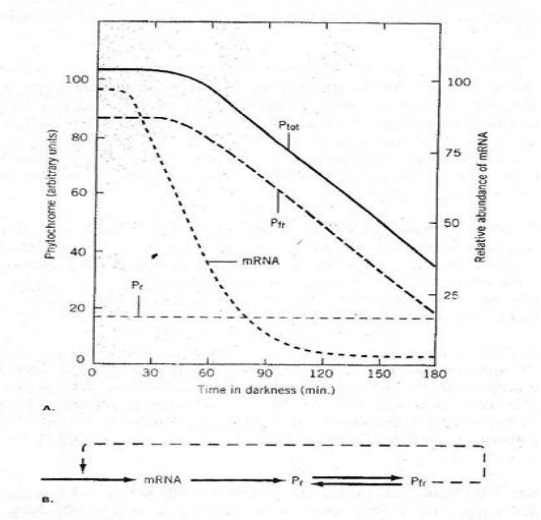

Fig. 7.10 A. Phytochrome-induced decline in phytochrome mRNA
B. The feedback inhibition of phytochrome mRNA by Pfr

In another interesting study, D. Ernst and D,Oesterheidt (1984) reported an increased transcription rate *in* v*itro* when phytochrome was added.to isolated rye nuclei. This suggests. The possibility of a direct interaction between phytochrome and the nucleus, obviating the need for an intervening protein or second messenger.

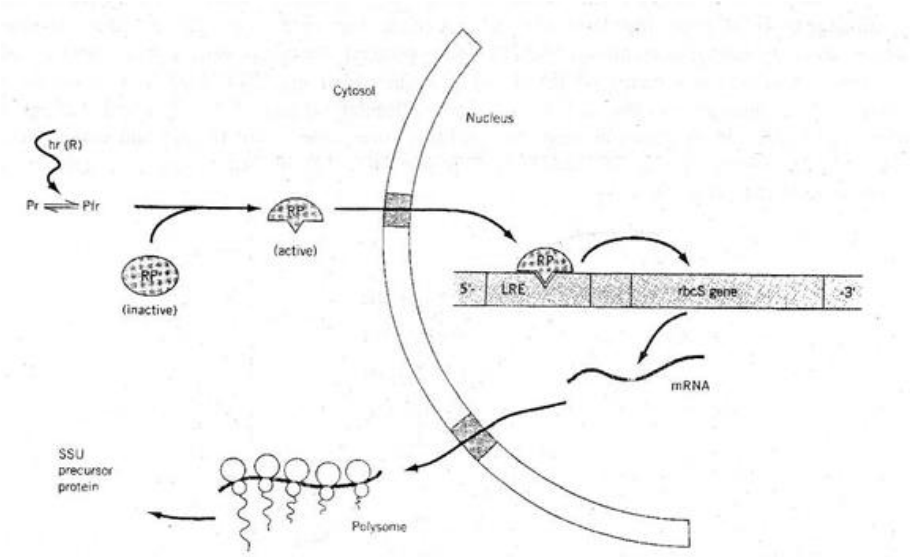

Fig. 7.11 Schematic model for phytochrome regulation of the Rubisco small subunit gene (rbcS)

There is still much to be learned about the molecular biology of development. The complex developmental events require the coordinated input of many gene products that must be expressed in the right tissues at the right time. When we understand something of these complex spatial and temporal interactions we will begin to understand and appreciate the true role of phytochrome in regulating plant responses to the natural radiation environment (Fig. 7.11).

Cryptochrome: A wide range of plant responses to blue and UV -A radiation have been known or suspected for a "long time. These responses are prevalent in lower plants such as ferns, mosses and fungi. These responses also share similar action spectra with higher plant responses such as photoperiodism and hypocotyl elongation. The identity of the blue/UV-A photoreceptor has proven difficult to unravel, hence the name cryptochrome, which means hidden pigment. Cryptochrome includes carotenoids and flavins or both. Like carotenoids, flavins are ubiquitous in living organisms. "The three most common are riboflavin, flavin mononucleotide (FMN) and flavin adenine dinucleotide (FAD). The flavins may occur free or complexed with proteins, they are called flavoprotein. Both FMN and FAD are important cofactors in cellular oxidation reduction reactions.

Using a combination of genetic, photobiological, and biochemical approaches, the Arabidopsis HY 4 gene has been shown to encode the blue photoreceptor, that mediates inhibition of hypocotyl elongation. The HY4 gene product is a cytoplasmic protein with a mass of about *7S* kDa and named CRY 1. The sequence of the CRY 1 protein is similar to photolyase, a flavoprotein that use blue light to stimulate repair of UV-induced damage to microbial DNA. Photolyases contain two chromophores, one flavin (FAD) and one a Pterin

(Fig. 7.12). CRYI appears to qualify as cryptochrome, at least with respect to inhibition of hypocotyl elongation in *Arabidopsis*.

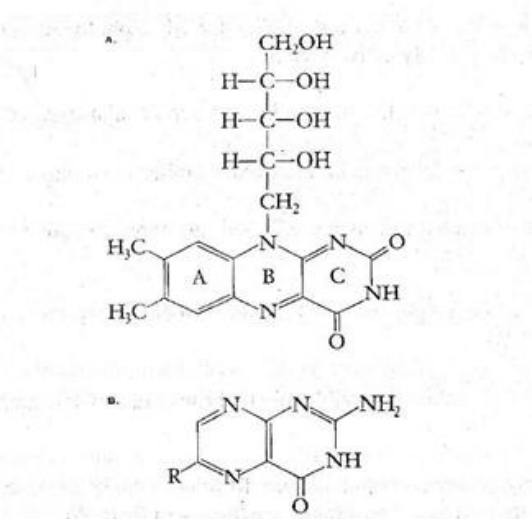

Fig. 7.12 The structure of Riboflavin (A) and Pterin (B).

7.4 Summary

- The phytochromes are a unique family of chromoproteins that play a critical role in almost every stage of plant development from seed germination-to flowering.
- The existence 'of phytochrome was predicted on the basis of physiological experiments that demonstrated pohotoreversibility with red (660 nm) and far red (730 nm) light.
- The pigment exists in two forms: PI' absorbs maximally at 660 nm and Pfr absorbs at 730 nm. When Pr absorbs far-red light, it is converted to Pfr, when Pfr absorbs far-red light, it is converted back to Pr.
- In *Arabidopsis* there are five phytochrome genes encoding five species of phytochrome (PHYA-E).
- Phytochrome A (PHY A) accumulates in dark grown seedlings as PrA, which is stable and
- PfrA is unstable and is destroyed with a half-life of 1 to 1.5 hours. PHYB is expressed at low levels in both light and dark.
- PfrB is stable with a half-life of 8 hours or more.
- A mixture of red and far-red light will establish a photo equilibrium mixture of P, and Pfr. Pfr is the physiologically active form.
- Phytochrome is a bluish chromoprotein with a molecular mass of about 124 kDa.
- A chromophore is an open chain tetrapyrrole similar in structure to phycocyanin.
- Phytochrome-mediated effects are grouped into three categories based on their energy requirements.
- Very low fluence responses (VLFR); low fluence responses (LFR) and high irradiance reactions (HIR).
- LFRs include photoreversible phytochrome responses such as seed germination and
- deterioration.
- VLFRs are not photoreversible and are difficult to study because they saturate at light levels below those that cause a measurable conversion of Pr to Pfr.

- HIRs require prolonged exposure to high irradiance, are time dependent and are not photoreversible.
- Phytochrome is the sensor that detects changes in red/far-red fluence ratio that occur under canopies and as end-of-day signals.
- The signal transduction pathway for phytochrome action is unknown.
- The identification of mutants deficient of one or more phytochrome is an important first step in deciphering this important regulatory system.

7.5 MODEL QUESTIONS

1. What is photomorphogenesis? Write about the historical aspects of photomorphogenesis.
2. Write an essay on phytochrome.
3. Discuss phytochromes in green plants.
4. Write short notes on:
   a) Cryptochrome
   b) Mechanism of Phytochrome action
   c) LFRs
5. Write an essay on physiological effects of phytochrome
6. Phytochrome and gene action.

7.6 REFERENCE BOOKS

1) Introduction to Plant Physiology, 2nd edition, William G. Hopkins, John Wiley & Sons, Inc.
2) Advanced Plant Physiology, Malcolm B. Wilkins, English Language Book Society/Longman.
3) Plant Physiology L. Taiz and E. Zeiger. Sinauer Associates, Inc., Sunderland, Massachussetts.

Dr. Madhuri vajha

LESSON -8

PLANT GROWTH REGULATORS

OBJECTIVES :

In this lesson, you will learn:

- A discussion of the hormone concept in plants and some of the controversies that surround it.
- An introduction of the five major groups of plant hormones - auxins, gibberellins, cytokinins, abscisic acid and ethylene - followed by a description of their principal physiological roles.
- A brief description of a few hypothetical hormones, the polyamines and other biological active substances including brassinosteroids that can influence growth and development.
- Description of two general models of hormone function. The hormone-binding proteins and their role in signal perception and of second messengers in the signal transduction pathway.
- Biosynthesis, transport and metabolism of auxins, gibberellins, cytokinins, abscisic acid and ethylene.

STRUCTURE OF THE LESSON:

8.1 INTRODUCTION

Multicellular plants are complex organisms, and their development requires an extraordinary measure of coordination between cells. To coordinate their activities, cells must be able to communicate with each other, often at some distance. The principal means of intercellular communication are hormones, the chemical messengers that carry information between cells and so coordinate their growth and development. Plant hormones have been the subject of intensive investigation.

The Hormone concept in plants: There are numerous chemical substances. natural and synthetic, that profoundly influences the growth and differentiation of plant cells and organs. Their role in development has been studied for nearly a century, yet the concept of hormones in plants are steeped in controversy.

In 1905, the British Physician E.H. Starling introduced the term hormone (Gr.; to excite or arose) to describe these chemical messengers. The concept of hormones in plants may be traced back to observations of Duhamel du Monceau in 1758. He observed the formation of roots on the swellings that occur above girdle wounds around the steins of woody plants. Root forming substances, produced in the leaves and migrating down the stem Would account for the initiation of roots above the wound. It was Darwin's' observations and experiments that ultimately led F.W. Went to describe a hormone-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 substances that, at low concentration, exert a profound influence on physiological processes. Like animal hormones, plant hormones naturally occurring organic substances that influence physiological processes at low concentration. The site of synthesis of plant hormones is not 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.

Whether plant hormones act in a concentration-dependent manner is a subject of continuing dispute among students of plant hormones. Some argue that plant cells respond to hormone concentration, as they do in animals; others argue that it is not the. Hormone concentration is important, but changing sensitivity of the target tells the hormone.

Another difficulty with plant hormones is the multiplicity of their effects. Each group of plant hormones is known to influence a wide variety of developmental events. Most of these events can be influenced by more than one hormone group (Table 8.1)

Table 8.1 The influence of plant growth hormone groups on different categories of development

(An x indicates a demonstrated effect of that hormone group on one or more aspects of that developmental category. The absence of an x does not mean that the hormone is ineffective, only that an effect has not been reported in literature).

	Hormone Group				
	Auxins	**Gibberellin**	**Cytokinin**	**Abscisic acid**	**Ethylene**
Dormancy		x	x	x	x
Juvenility	x	x			
Extension Growth	x	x	x	x	x
Root development	x	x	x		x
Flowering	x	x	x	x	x
Fruit development	x	x	x	x	x
Senescence	x	x	x		x

The differences between animal and. plant hormones led to some confusion in terminology. The plant growth. substances are preferred by some-there is even an International Plant Growth Substance Society. Others argue that "substances" too vague ..and growth.is influenced by these chemicals. A second term, plant growth regulator is preferred, and it is used to denote synthetic compounds that exhibit hormonal activity.

There are currently five recognized groups of plant hormones: auxins, gibberellins, cytokinins, abscisic acid (ABA) and ethylene. In addition to these hormones, two other groups appear to be active in regulating plant growth, the brassinosteroids and polyamines.

8.2. AUXINS

Auxins were the first plant hormones to be discovered. Auxins are synthesized in the stem and root apices and transported through the plant axis. They are characterized principally by their capacity to stimulate cell elongation in excised stem and coleoptile sections but also influence a host of other developmental responses, including root initiation, vascular differentiation, tropic responses and the development of axillary buds, flowers and fruits.

8.2.1 Natural and synthetic auxins

The principal auxin in plants is indole-3-acetic acid (IAA) (Fig. 8.1). Indole-3-ethanol, indole-3-acetaldehyde and indole-3-acetonitrile are also naturally occurring auxins. All these serve as precursors to IAA and their activity is due to conversion to IAA in the tissue. The synthetic chemicals also express auxin like activity. One of these, indole butyric acid (IBA), was originally thought to be strictly synthetic, but recently IBA has been isolated from seeds and leaves of maize and other species (Epstein et al., 1989). A chlorinated analog of IAA (4-chloroindolacetic acid, or 4-chloro IAA) has also been reported in extracts of legume seeds (Engvild, 1986) and phenylacetic acid (PAA) (Fig. 8.2A, B), a naturally occurring aromatic acid, has recently been reported to have auxin activity (Leuba and Le Toureau, 1990). It has not yet been established whether or not they are converted to IAA in the tissue before they become active.

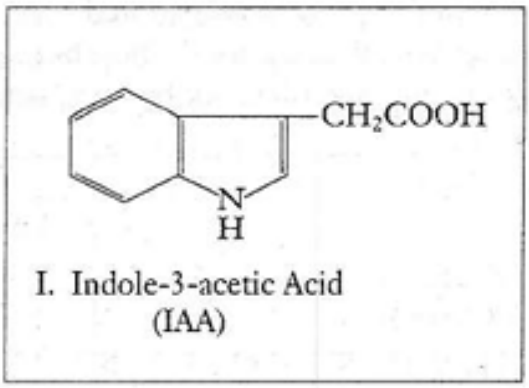

Fig. 8.1 The true auxin in plants, IAA

8.2.2 Biosynthesis and metabolism of auxins

The auxin IAA is ubiquitous in the plant. Highest concentrations of the hormone are detected in meristematic regions and actively growing organs like coleoptile apices, root tips, the apical buds of growing stems and germinating seeds. The major IAA synthesis sites are young, rapidly growing leaves, developing inflorescences and embryos following pollination and fertilization.

The amount of IAA present depends on the type and age of tissue and its state of growth. For example, in vegetative tissues, the amount of IAA is between 1 µg and 100 µg kg-1 fresh weight, but in seeds it appears to be much higher. The high level of hormone in seed helps in the growth of young seedlings when the seed germinates.

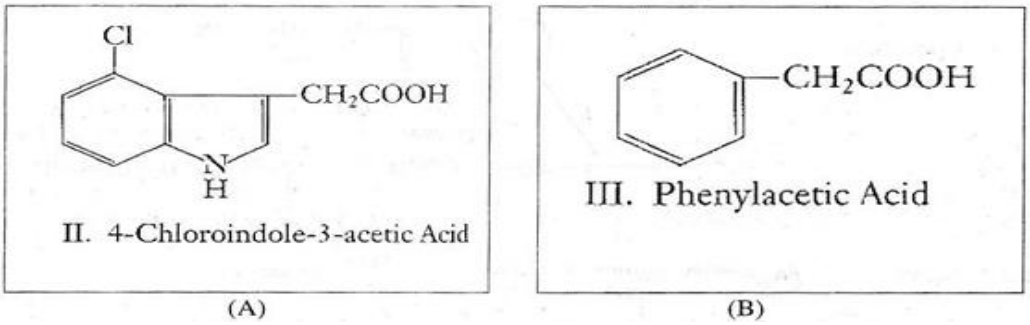

Fig. 8.2 A) 4-chloroindole-3-acetic acid; B) Phenylacetic acid

8.2.3 Biosynthesis of IAA

IAA is synthesized from the aromatic amino acid, tryptophan. In 1930, K.V. Thimann first observed the synthesis of IAA in the mold *Rhizophus suinus,* which had been fed the amino acid tryptophan, which is converted to IAA. The synthesis of IAA is studied by feeding the plants tryptophan carrying a radioactive label, usually carbon (^{14}C) or tritium (^{3}H) and examined the radioactivity of isolated IAA or its intermediates.

- In most higher plants, synthesis of IAA occurs in three steps, conversion of tryptophan to indole-3-pyruvic acid (IPA) (Fig. 8.3). This transamination reaction is catalyzed by tryptophan amino transferase, this will remove amino groups from tryptophan, phenylalanine and tyrosine.
- The second step is the decarboxylation of IPA to form indole-3-acetaldehyde (IAAld) in presence of an enzyme indole-3-pyruvate decarboxylase.
- Final step is, IAAld is oxidized to IAA by a NAD-dependent indole-j-acetaldehyde dehydrogenase.
- IAAld may also be reversibly reduced to indole-3-ethanol. This exhibits auxin activity in bioassays of stem sections.

In Arabidopsis and in members of Brassicaceae, an alternative pathway is evidenced IAA may also be formed from indole-3-acetonitrile (IAN), an indole derivative. IAN exhibits auxin activity, probably by conversion to IAA through the action of a nitrilase enzyme.

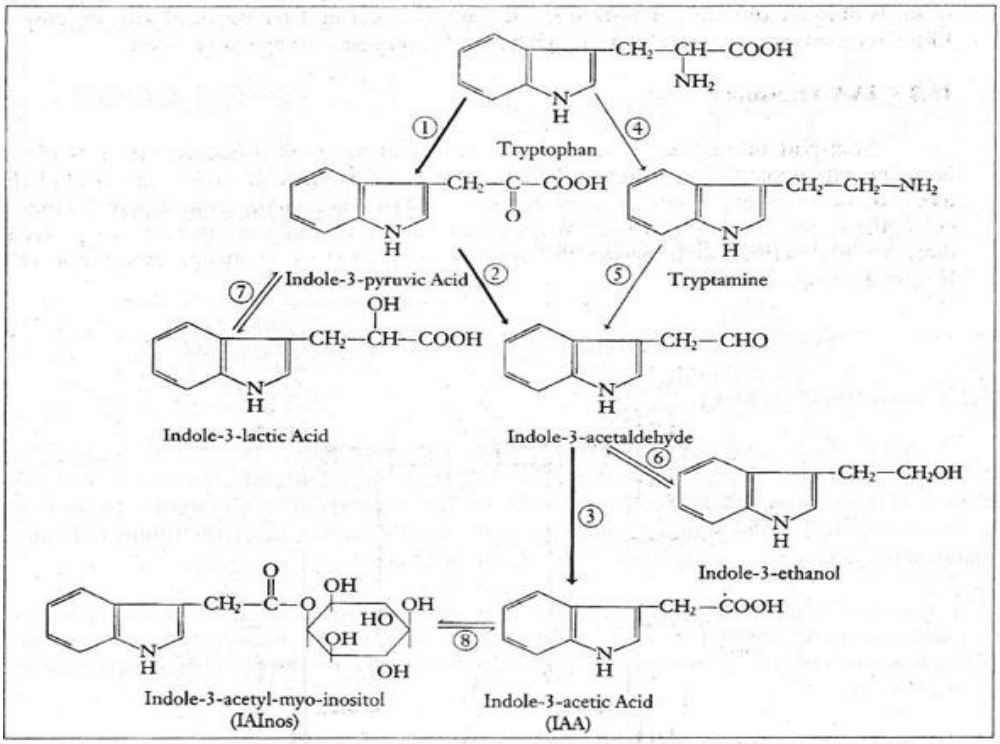

Fig. 8.3 The biosynthetic pathway of Auxin (IAA)

8.2.4 IAA conjugates

Two populations of the hormones were recognized. One was free moving, could be obtained by diffusion into Agar, the other is bound auxin, could be obtained by solvent extraction or

by hydrolysis under alkaline conditions. The bound auxin is now recognized as IAA that has formed chemical conjugates like glycosyl esters or peptides.

IAA conjugates are inactive but release free, active IAA upon solvent-extraction, alkaline hydrolysis or in vivo enzymatic hydrolysis. In germinating seedlings, large pools of IAA conjugates appear to be important source of active hormone.

The site of synthesis of IAA conjugates are not well understood. The conjugates found in seeds of Zea mays are synthesized in the seed itself during later stages of seed development. Little information is known about the synthesis of conjugates in vegetative tissues.

8.2.5 IAA Transport

Transport into or out of a tissue or organ will naturally influence the level of active hormones within that tissue or organ. Polar transport is an example of auxin transport. Polarity in auxin transport is expressed as a preferential movement of auxin from top to bottom in a coleoptile or shoot axis (Fig 8.4). When the movement is from apex to base of the plant, the direction is described as basipetal; the opposite is referred as acropetal. Roots also exhibit basipetal transport of auxin.

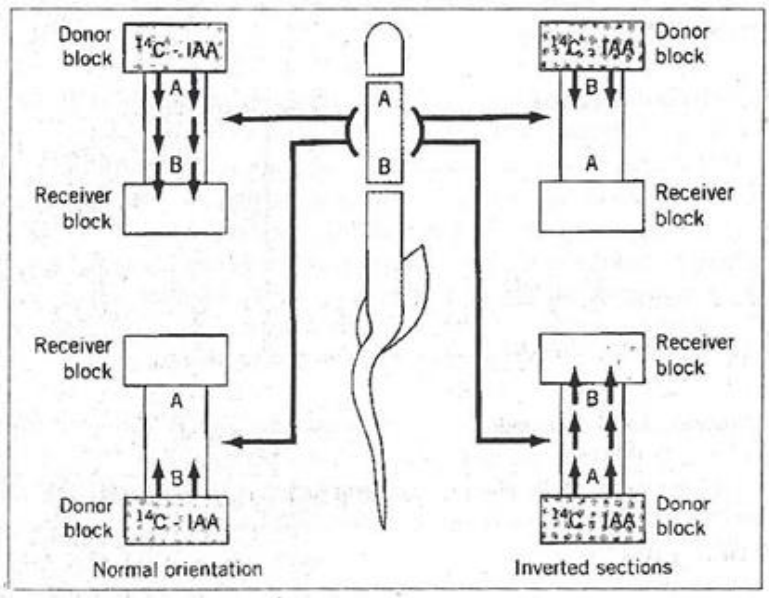

Fig: 15~4Polarity in auxin transport in an oat coleoptile segment

How is polarity in auxin transport established? Many observations indicate the involvement of some form of carrier-mediated, active transport mechanism. First, it can be shown that polar transport is inhibited by respiratory poisons such as cyanide and 2,4-dinirophenol.

Second, certain chemicals called phytotropins, like 2,3,5-triiodobenzoic acid (TIBA), 9-hydroxyfluorine-9-carboxylic acid (HFCA or morphactin) and N-l-naphthylphthalmic acid (NPA).

Third, the uptake of radioactive IAA is partially inhibited by non-radioactive IAA. These results suggest that the labelled and unlabeled IAA compete for a limited number of carrier sites.

IAA is a weakly acidic, lipophilic molecule. Depending on the pH, IAA may exist either in the protonated (IAAH) or the unprotonated, anionic form (lAA). In cell wall space, where pH is about 5.0, any IAA- will rapidly protonate to form IAAH. IAAH has higher lipid solubility and penetrates more readily than IAA. It has been confirmed that the uptake of IAA into cells increases as the extracellular environment is made more acidic. Thus, cells will take up auxin from the cell wall space as IAAH diffuses down its concentration gradient. In cytoplasm, where pH is about 7.0, IAAH will dissociate to lAA and H+. The pH difference between the cell wall space and the cytoplasm serves to maintain the IAAH concentration gradient and encourage IAAH to continue moving into the cell.

More recently, inhibitors of polar transport are provided to be useful tools for exploring the role of auxins in developmental' phenomena. One example is early stages in the formation of flower buds in Arabidopsis. Pin i is a mutant of *Arabidapsis* that results in abnormal floral development. Polar transport of auxins is also affected by the Pin l mutation. The polar transport of exogenously supplied ^{14}C-labelled IAA is reduced to 10% of normal. The interesting thing here is that the mutant phenotype could be generated in wild-type seedlings by applying the auxin transport inhibitors HFCA and NPA. Another auxin antagonist (2-p-chlorophenoxyl - isobutyric acid or (PIB) is known to inhibit auxin activity but does not interfere with polar transport and does not generate the mutant phenotype.

In another study, Liu and coworkers have shown that HFCA stimulates the formation of fused cotyledons in cultured mustard embryos. HFCA interferes with the normal initiation of two cotyledons and the transition from the axial symmetry of the early globular shaped embryo. Both studies show how inhibitors of auxin transport can be used as developmental probes.

8.2.6 Oxidation of IAA

IAA in aqueous solution is degraded by acids, ultraviolet, ionization radiation and visible light in presence of sensitizing pigments such as riboflavin. The most prevalent form of IAA degradation appears due to oxygen and peroxide, either separately or in combination, in the presence of a suitable redox system.

An enzyme responsible for inactivating IAA was first isolated from plant extracts by Tang and Bonner (1947) and was called IAA oxidase. Oxidative decarboxylation of IAA is known to be catalyzed by peroxidases from a variety of plant sources. The oxidative decarboxylation of IAA by peroxidase is now recognized by some physiologists to be synonymous with IAA oxidase.

The pathway for oxidative decarboxylation of IAA is shown in Figure 8.5. The major end products of oxidation by cell-free enzyme preparations are 3-hydroxy-methyl oxindole and 13-methyleneoxindole. IAA oxidase activity is higher in the older, non-growing tissues than it is in younger, actively growing tissues, which have a high auxin requirement. Oxidative break Idow is the only known means for irreversibly removing IAA from the active pool and may be very important in regulating IAA-mediated responses.

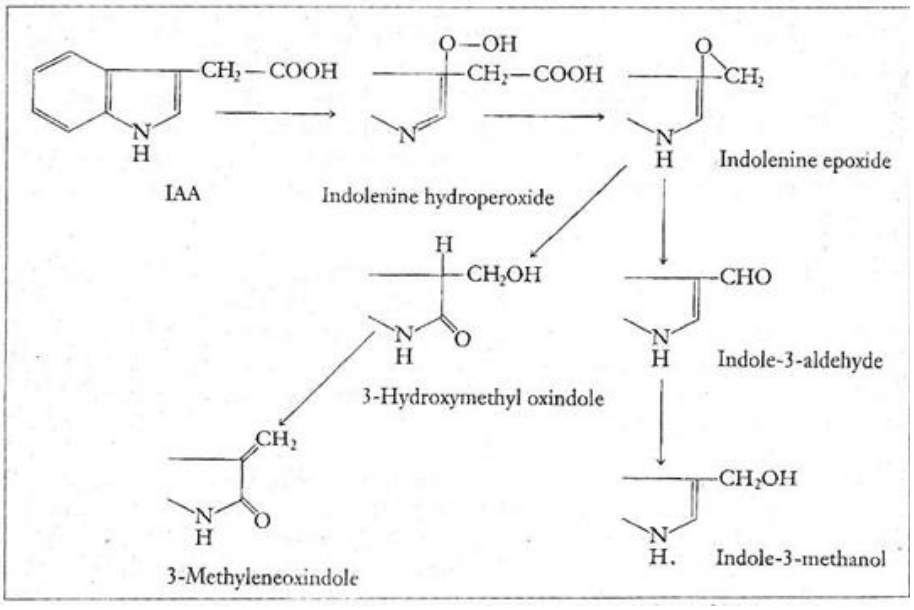

Fig. 8.5 Schematic pathway for oxidative degradation of IAA

8.2.7 Mechanism of action of auxins

The primary mechanism of hormone action in plants generally and auxin continues to elude us more than half a century after its discovery. Although auxin appears to be evolved in a wide range of growth and developmental responses, efforts to understand how auxin works have focused largely on the fundamental role of auxin in cell expansion.

Auxin and cell expansion: Cell expansion is the most studied hormonal response in plants. Two major theories have been proposed to account for auxin-induced cell expansion. In the 1960s, shortly after Watson and Crick's DNA structure and DNA-RNA-protein dogma, it was proposed that auxin activated the genes for certain proteins that were necessary for all growth. In the meanwhile, Mitchell's chemiosmotic model for oxidative phosphorylation was gaining universal acceptance and interest was turning toward cellular membranes and the control of ion flux across membranes - and ATPase proton pumps. A second theory, which attributed cell expansion to auxin-induced proton excretion, was the acid growth theory.

An increase in growth rate would require an increase in wall extensibility (m), an increase in turgor pressure (P) or a decrease in yield threshold (Y). Direct measurements of P, using a micro pressure probe, indicate that turgor pressure does. not change during auxin stimulated increase in the growth rate of pea stem sections. Although Y cannot be measured directly, the results of indirect tests indicate that yield threshold does not change either. That leaves extensibility, (m) and extensibility is difficult to assess. It is on the one hand a rate coefficient, but it is also a measure of the capacity of cell walls to undergo irreversible (plastic) deformation. A change in m should be reflected as a change in the physical properties of the wall, especially plasticity or its capacity to undergo permanent deformation. So there is a general agreement that induction of rapid cell enlargement by auxin is accompanied by large increases in m. It is concluded that auxin stimulates cell expansion by increasing wall extensibility.

Acid Growth theory: In 1970, D. Rayle and R. Cleland suggested that auxin causes acidification of the cell wall environment by stimulating cells to excrete protons. There the

lower pH activates one or more wall loosening enzymes. At about the same time, A. Hanger, in Germany, proposed that auxin stimulated proton excretion by activating a: plasma membrane bound ATPase proton pump. The combined Cleland-Hager proposals, known as the acid growth theory, are summarized in Fig. 8.6.

Plant membranes contain ATPase enzymes that catalyze the electrogenic transport of protons. It is important to note that auxin-binding proteins do not exhibit ATPase activity. It is unlikely that the plasma membrane ATPase is itself the auxin receptor. Still, auxin causes hyperpolarization of the cell membrane beginning about 8 to 10 minutes after auxin application. Hyperpolarization of the membrane would result from the activation of an electrogenic ATPase proton pump.

Auxin will also cause growing cells to excrete protons. This is an energy dependent process. Metabolic inhibitors and inhibitors of auxin induced growth will inhibit auxin-induced proton excretion. With Avena coleoptiles, the pH of the apoplastic solution drops from 5.7 to 7.7 within 8 to 10 minutes of auxin application. Acid solutions at a pH of3. 4 are required to induce a rate of elongation comparable to optimum auxin concentrations. Auxin also activates phosP9olipase A_2 (PLA_2), a membrane bound enzyme that hydrolytically excises the fatty acid from the central (C_2) glycerol carbon of a phospholipid. The product is a free fatty acid plus a phospholipid with a single fatty acid, called a lysophospholipid (LPC). Activation of PLA2 can be blocked by Ig G-antiABP (Auxin binding protein) and the products ofPLA2, both LPC and fatty acids, stimulate proton secretion and elongation.

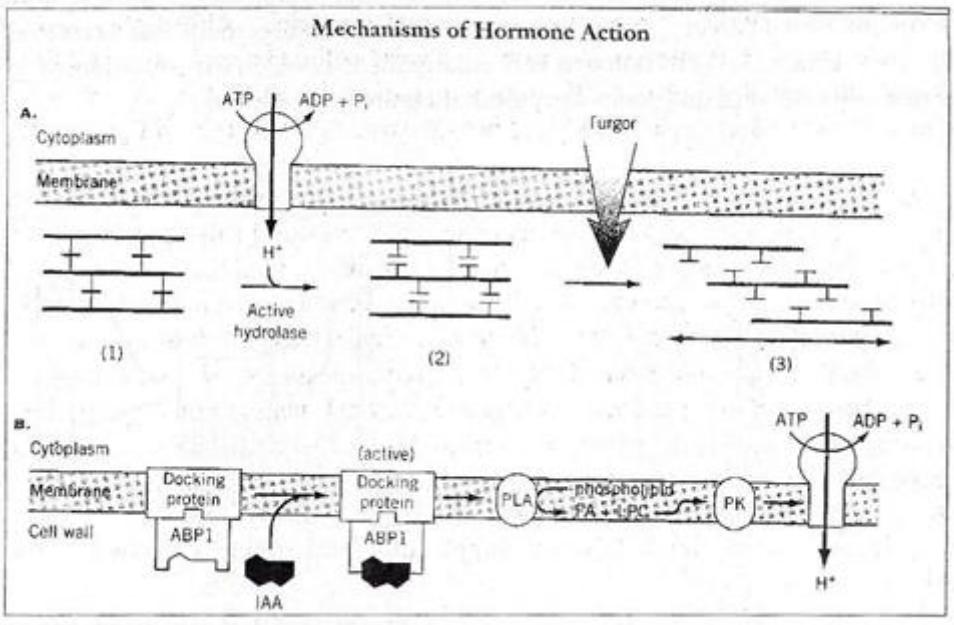

Fig. 8.6 The acid growth hypothesis for cell enlargement. (A) Cell wall polymers are extensively cross-linked with load-bearing bonds (I), which limits the capacity of the cell to expand. An ATPase proton pump located -in the plasma membrane acidifies the cell wall space by pumping protons from the cytoplasm. The lower pH activates wall-loosening enzymes that cleave the load- bearing bonds (2). The force of turgor acting on the membrane and cell wall cause the polymers to auxin with activation of the ATPase proton pump. See text for details. Abbreviations: ABP 1, auxin binding protein 1; PLA, phospholipase A2; FA, fatty acids; LPC, lysophospholipid; PK, protein kinase. (B based on Macdonald, 1997).

These effects are inhibited by vanadate, which blocks the. plasma membrane proton-ATPase. These data suggest that PLA_2 follows ABPI in the chain and that lipids, both LPC and fatty acids, function as second messengers. Both the IAA and LPC effects on proton secretion and elongation can be blocked by protein kinase inhibitors, suggesting that the lipids activate the proton-ATPase by a phosphorylation dependent mechanism.

8.2.8 Physiological action of Auxin

(a) Cell growth and differentiation: Auxin regulated cell elongation in Avene coleoptiles was the' basis for its discovery. Auxin concentration-response curves show that response will be increased with increased concentrations of auxin. till an optimum concentration is reached (Fig. 8.7). Concentration exceeding the optimum results in reduced growth. Growth responses are often said to assay for unknown hormone concentrations, a technique known as bioassay. Intact stems and coleoptiles do not show a significant response to exogenous auxin application. High endogenous auxin content of intact tissues support maximum elongation and the added auxin has little or no additional effect. Auxin is essential for cell enlargement and growth of leaves, flowers and other organs. Auxin-induced cell enlargement is basis for initiation of growth of undifferentiated cells when plant tissues are cultured on artificial media.

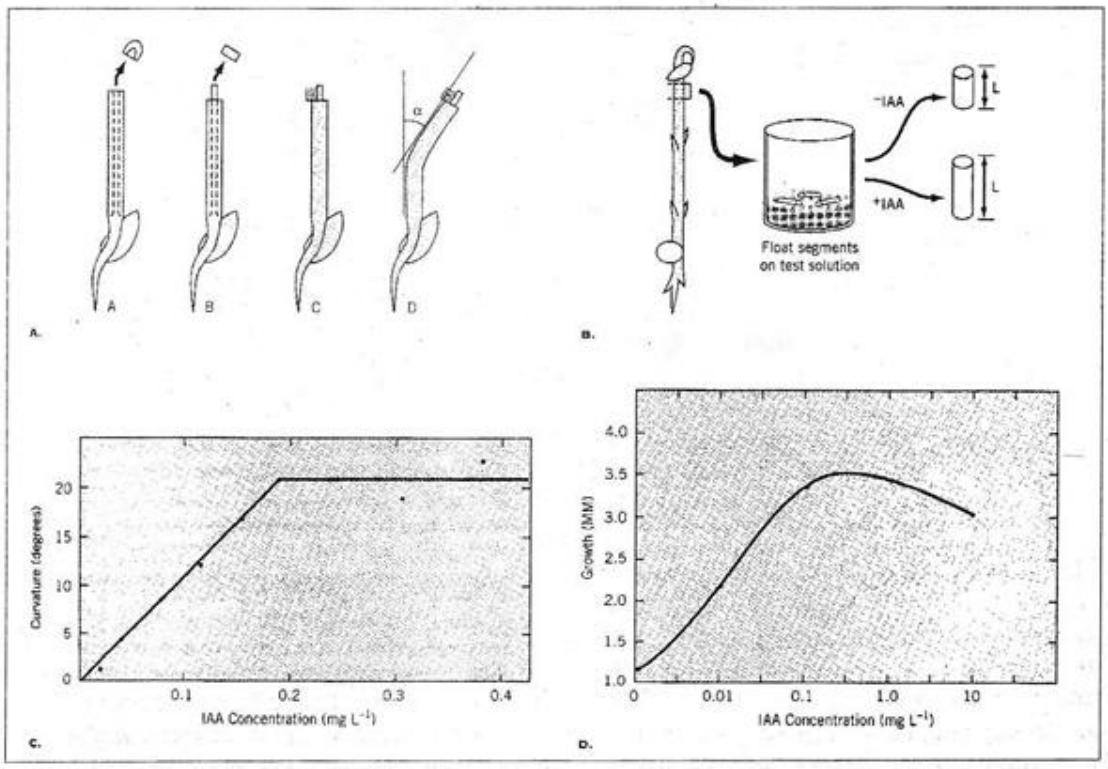

Fig. 8.7 Concentration response curves for two classic auxin responses

Auxins also induce vascular differentiation in young and rapidly developing leaves. W.Pi.Jacobs and coworkers found that regeneration of vascular tissues around wounds in Coleus (Lamiaceae) is also under the control of auxin. The extent of vascular regeneration is directly proportional to the auxin supply when exogenous auxin is substituted for the leaves, as leaves are the sources of auxin.

Auxin is also required for vascular differentiation in plant tissue culture. When buds (source of auxin), are' implanted into clumps of undifferentiated callus tissue in culture,

differentiation of callus parenchyma into vascular tissue occurs in regions adjacent to the implant. The same effect is achieved when agar wedges containing IAA and sugars are substituted. for the implanted bud.

Shoot and root development: There are two types of buds, axillary buds and apical buds. In many plants, mitosis and cell expansion in the axillary bud are arrested at an early stage and the bud fails to grow. The removal of shoot apex stimulates the axillary bud to resume growth. The apical but can exert a dominant influence that suppresses cell division and enlargement in the axillary bud. The phenomenon of coordinated bud development is known as apical dominance.

How does auxin from the shoot apex suppress axillary bud development? The most widely accepted theory is that the optimum auxin concentration for axillary bud growth is much lower than it is for the elongation of stems. Auxin flows out of the shoot apex to the base of the plant is thought to maintain an inhibitory concentration of auxin at the axillary bud. Removal of this auxin supply by decapitation reduces the supply of auxin in the region of the axillary and thereby relives the bud of inhibition. Inhibitors of auxin transport triiodobenzoic acid (TIBA) and naphthylphthalamic acid (NPA), stimulate release of buds from dominance when applied to the stem between the shoot apex and the bud. Lines of tomato that exhibit prolific branching (in absence of apical dominance) also fail to export radioactively labelled IAA from the shoot apex.

It is now clear that cytokinins will antagonize the auxin effect. Application of cytokinins either to stem apex or to the axillary bud will release the bud from inhibition.

Other experiments have shown that, there is a relation between the inhibition of bud growth and Abscisic acid (ABA) content of the bud. It appears that the ABA content in the axillary bud is under control of IAA moving down from the shoot apex. Application of ABA to the shoot apex also releases axillary buds from inhibition. Ethylene production, stimulated by auxins, has also been implicated in axillary bud inhibition, but there is no conclusive evidence. The nature of other hormonal interaction with auxin is complex and has not been clearly defined.

Leaf Abscission: The process of shedding organs is known as abscission. Abscission occurs as a result of the development of a special layer of cells called the abscission layer. As the organ ages, the cell. walls in the abscission layer weaken and separate. Abscission appears to be dependent on the concentrations of auxin on either side of the abscission layer. Auxin content is high in young and rapidly growing portions of a plant and decline as the organ ages and approaches senescence. This can be demonstrated by excising a leaf blade while leaving the petiole attached to the stem. If auxin is applied to the cut end, distal to the abscission layer, abscission of petiole will be delayed when compared with the controls.

Root elongation and development: Root elongation is sensitive to auxin. At low concentrations (10^{-8} M or less) IAA will promote the growth of excised root sections and intact roots. Higher concentrations (10^{-5} to 10^{-6} M) cause inhibition of root growth. This indicates that high auxin concentration stimulates production of ethylene. Removal of root tips or application of auxin antagonists promotes the growth of roots. Auxin inhibits root elongation, but high auxin concentrations will promote initiationof secondary roots. Removal of young

leaves and buds (sources of auxin), reduce the number of secondary roots formed. Auxins also promote adventitious root formation on stems (Fig. 8.8).

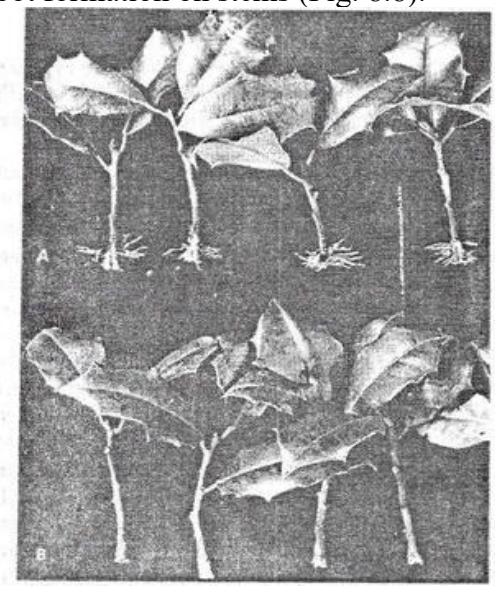

Fig. 8.8, Auxin stimulated adventitious root development
A. Treated with Indole Butyric acid
B. Untreated controls

Flower and fruit development: Auxin does not seem to play a major role in the initiation of flowers, since exogenous auxin tends to inhibit flower formation. But members of the family Bromeliaceae exhibit a strong stimulation of the flowering response following application of either auxin or ethylene.

The sex of imperfect flowers (monoecious or dioecious) is genetically determined. In the cucurbits the flower is bisexual in its early stages but one sex organ or the other aborts. Application of auxin during the bisexual stage ensure the formation of female flowers. Fruit set, i.e., the initiation of ovary development requires successful pollination and fertilization. During the mid-1930s, it was found. that pollen was a rich source of auxin. The synthetic auxin, 4-chlorophenoxy-acetic acid is used to stimulate fruit set, when cool night temperatures would tend to reduce fruit set, Auxin induces parthenocarpy. J.P. Nitsch in the 1950s indicated that the developing seed was the source of auxin for continuing fruit development. Nitsch found that removal of seeds prevented further development of the fruit, but supplying the fruit with auxin restored normal development. Auxins may also be used to control abscission of fruits. Auxins may cause early fruit drop or prevent premature fruit drop. This causes an increase in the size of those remaining on the tree, which as thinning of fruits. Later application of auxin delays abscission thus preventing premature fruit drop.

8.3 GIBBERELLINS

Gibberellins are produced both by fungi and higher plants. The exogenous application of gibberellins causes hyper elongation of intact stems. Gibberellins are also involved in seed germination and mobilization of endosperm reserves during early embryo growth and also flower and fruit development.

Gibberellins are the only hormones that can be defined based on their chemical structure than biological activity. The chemical family of this hormone is based on the ent gibberellane structure (Fig. 8.9). Based on the number of carbon atoms in the structure, gibberellins are known as C_{20}-gibberellins and C_{19}-gibberellins. The naturally occurring gibberellins have been chemically characterized are assigned as "A" number. GA_3, a C_{20} gibberellin also known as gibberellic acid (GA) was the first isolated and characterized gibberellin from fungal cultures. GA_1 and GA_{20} are C_{19} - GAs, the most important gibberellins in higher plants. A carboxyl group at carbon-7in all GAs is required for biological activity. C_{19}-GAs are more biologically active than C_{20}-GAs. Those GAs with 3-β-hydroxylation, 3-β, 13-dihydroxylation or 1,2-unsaturation are more active and those with both 3-β-0H and 1,2-unsaturation exhibit the highest activity.

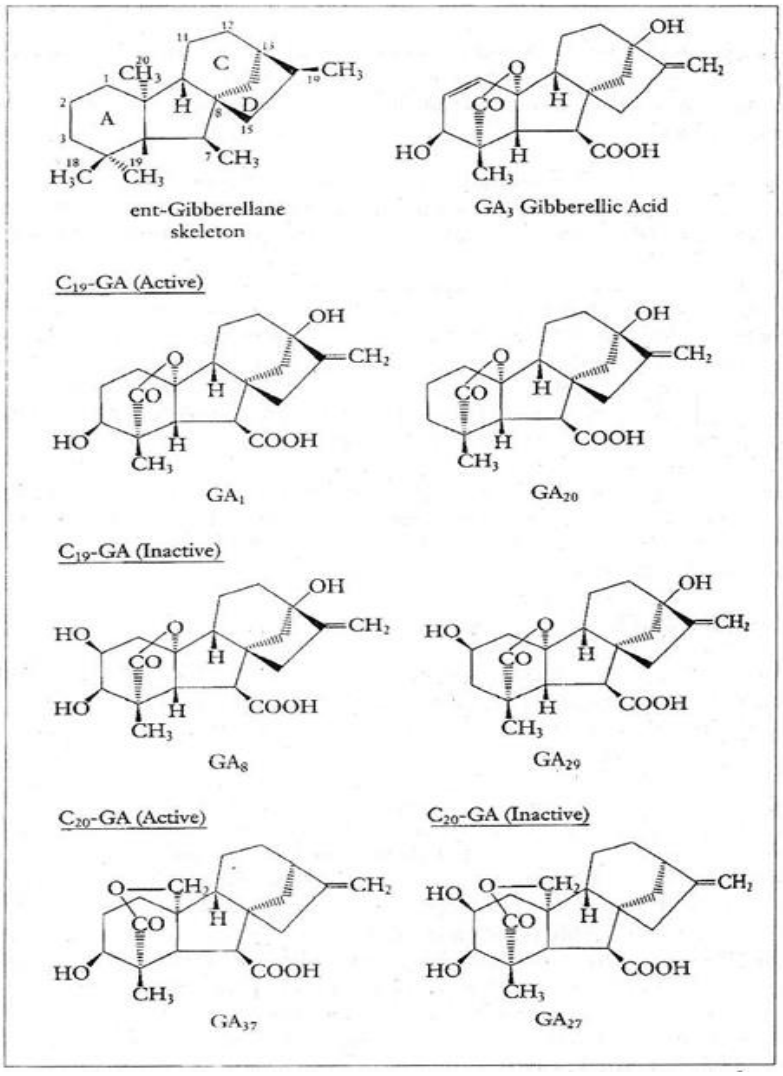

Fig. 8.9 The ent-gibberellane skeleton and chemical structures of selected active and inactive gibberellins

8.3.1 Gibberellin biosynthesis and metabolism

Developing seeds, fruits, the young leaves of developing apical buds and elongating shoots and the apical regions of roots are the sites of gibberellin biosynthesis. Immature seeds and fruits are prominent sites of gibberellin biosynthesis. As the seeds mature, metabolism appears to shift in favor of gibberellin-sugar conjugates.

8.3.2 Gibberellic acid biosynthesis

Gibberellins are diterpenoid acids related to naturally occurring terpenoids. Gibberellin synthesis may be considered in three stages. The first stage is the mevalonic acid pathway, which leads to other terpenoids. In this pathway, the 5-carbon (C_5) isoprenoid unit isopentenyl pyrophosphate (IPP) is synthesized from acetyl coenzyme A and used to build up the C_{20} geranylgeranyl-pyrophosphate (GGPP).

The second stage in the synthesis of gibberellins is the biosynthesis of ent-kaurene from GGPP and its conversion to GA_{12}-7-aldehyde. GA_{12}-7-aldehyde is the precursor for all other known gibberellins. The third stage is the biosynthesis of all gibberellins from GA_{12}-7-aldehyde (Fig. 8.10).

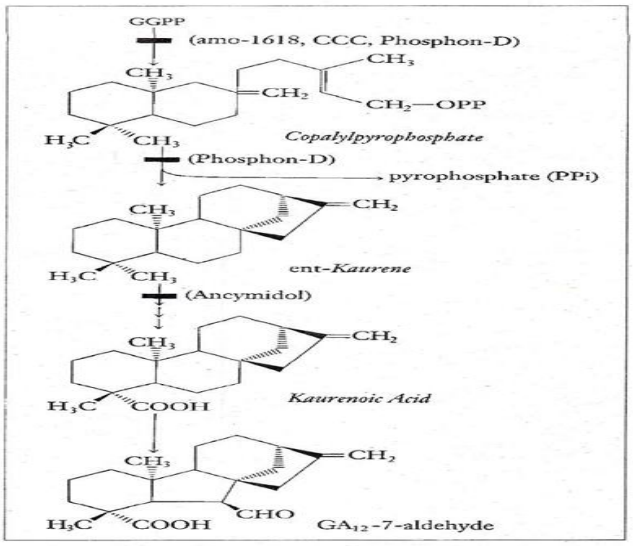

Fig. 8.10 Gibberellin biosynthesis

The first two steps in the synthesis of gibberellins from GGPP involve the cyclization of GGPP first to copalylpyrophosphate, then to kaurene. Flowing cyclization, the carbon at 19 positions on kaurene undergoes three successive oxidations in the sequence $CH_3 \rightarrow CH_20H \rightarrow CHO \rightarrow COOH$ to form kaurenoic acid. The two cyclization steps and the oxidation kaurene to kaurenoic acid are inhibited by antigibberellin dwarfing agents like Amo-1618, CCC, phophon-D and ancymidol. The final two steps involve a hydroxylation of carbon-7 and contraction of the ring with extrusion of carbon-7 to form GA_{I2}-7-aldehyde. Oxidation of aldehyde group on carbon-7 to carboxyl group gives GAI2. The CI9-GAs arise by subsequent oxidative elimination of carbon-20. Interconversions among GAs in pea seed and seedlings are presented in (Fig.8.11). The 13-hydroxylation pathway (bold arrows), leading to GA_{20} and $GA_{l,}$ is probably of widespread occurrence in higher plants.

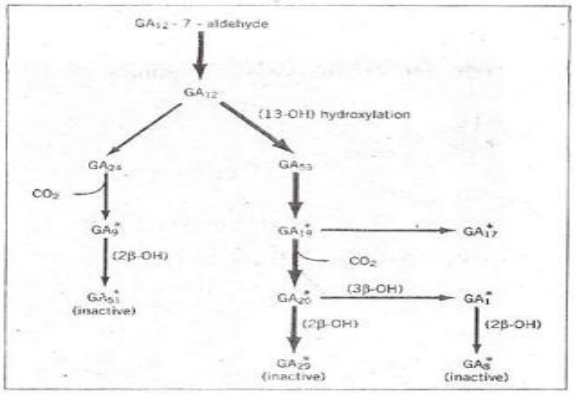

Fig. 8.11 Proposed pathways for gibberellin biosynthesis in pea

In immature, actively developing seeds, the principal free gibberellins are GA_1, GA_8. Small amounts of GA_4, GA_5, GA_6 (C_{19} GAs) and GA_{37} and GA_{38} (C_{20} GAs) are also found. Mature seeds contain GAs-glucoside and small amounts of GA_1, GA_4, GA_{37} and GA_{38} glucosyl esters.

Gibberellins have been detected in both the phloem and xylem saps, studied by conducting the- application of radioactively labelled GAs to stem or coleoptile sections. Transport is not to be polar but moves along with other phloem-translocated organic materials according to a source-sink relationship. Gibberellins synthesized in the root tips are distributed to the aerial portions of the plant through the xylem stream. It is not known that gibberellins are transported as free hormones or in the conjugated form.

8.3.3 Mechanism of Gibberellin action

1) Gibberellin control of stem elongation: Gibberellins act to stimulate both cell division and cell elongation in stems. In rosette plants, the rapid elongation is accompanied by cell divisions in the region just below the apical meristem and hyper elongation of daughter cells following gibberellin application.

The gibberellin response in lettuce hypocotyls, is a reversal of blue and far-red light inhibition. Dwarf pea and cucumber stems are inhabited by red light (Fig. 8.12). This inhibition is also reversed by gibberellin.

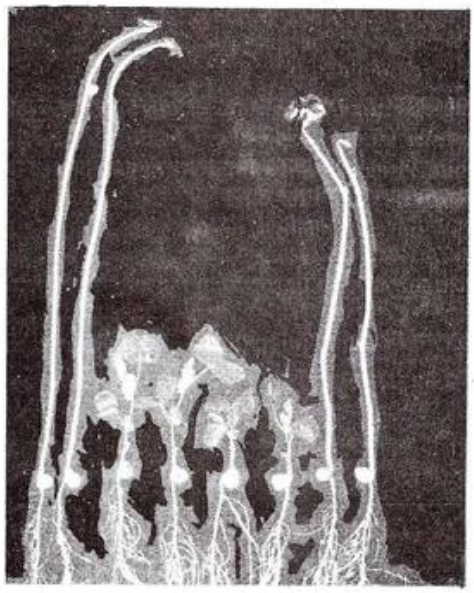

Fig. 8.13 Dwarf pea and cucumber stems are inhibited by GA

2) GA control seed germination: The ά-amylase secreted from barley aleurone consists of multiple isozymes that fall into two major families characterized by their isoelectric points (p^1). The low group has pls in the range of 7.5 to 7.85 and the high group has pIs in the range of 5.9 to 7.3. Within each group, the isozymes are quite similar but between the two groups there are major differences with respect to calcium requirement for secretion and sensitivity to ED~A and heavy metals. The two groups of isozymes are translated in vitro from two different mRNA populations encoded by two multigene families located on different chromosomes.

In addition to ȧ-amylase, proteolytic enzymes (proteases), β-amylase and other starch degrading enzymes are involved in mobilizing the endosperm reserves (Fig. 8.13): stimulated ex-amylase synthesis IS inhibited by inhibitors of transcription and that GA induces significant changes in RNA metabolism, especially mRNA. If GA acts to regulate gene expression, it clearly must regulate a large number. of genes from several different families spread throughout the genome.

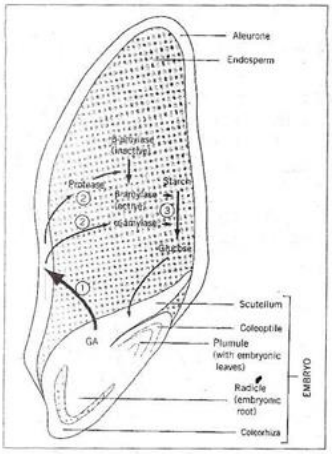

Fig. 8.13 Gibberellin induced release of enzymes and carbohydrate mobilization during germination.

Does GA regulate transcription of ȧ- amylase mRNA? Based on evidence from several lines of investigation, gibberellin dose regulates transcription of ȧ-amylase mRNA. Both ill vivo pulse labeling of protein and in vitro translation of protein from total aleurone RNA, followed by electrophoretic and autoradiographic analysis, show significant increase in the amount of ȧ-amylase translated following the application of gibberellin (Fig. 8.14). Following gibberellin treatment, ȧ-amylase mRNA may comprise 20% of the total translatable mRNA. Finally, the rate of a amylase synthesis following gibberellin treatment closely correlates with the rate of mRNA accumulation.

Fig.8.14 Hormonal control of ȧ-amylase biosynthesis by barley alemon layers

8.3.4 The Physiological action of Gibberellins

1) Control of shoot elongation - Dwarf plants: It was excessive stem elongation in infected rice plants that led to the discovery of gibberellins and hyper elongation of stem tissue is one of the effects of gibberellins on higher plants. The relationship between dwarfing or internode length genes and gibberellins was pioneered by the work of B.O. Phinney on maize (Zea mays) and P.W. Brain and coworkers on garden pea (*Pisum sativum*). Application of exogenous gibberellin to the dwarf mutant of rice, bean, Arabidopsis thaliana and several others restore a normal, tall phenotype (Fig. 8.15). Exogenous gibberellins have no effect on the genetically normal plant.

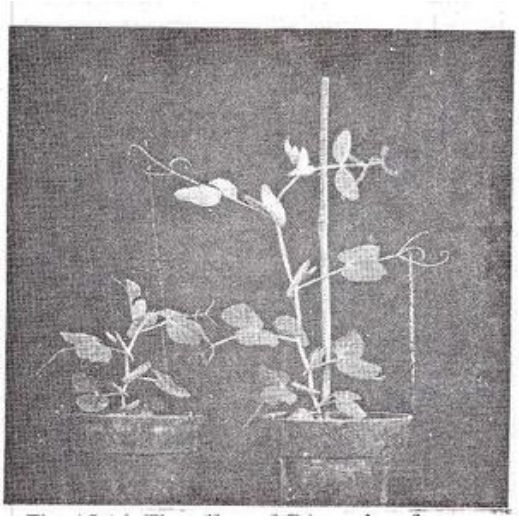

Fig. 8.15 The effect of GA on dwarf pea

There are five mutants in maize, d_1, d_2, d_3, d_5 and an_i, exhibit the normal phenotype when treated with GA3, but show no response to other hormones or growth regulators. While studies with dwarf plants have been instrumental in linking gibberellins. As with stein elongation, there are' other dwarf mutants known that do not respond to application of gibberellin.

2) Rosette Plants: A rosette is an extreme case of dwarfism. In this case, there is an absence of internodal elongation which is characterized by closely spaced leaves. The failure of internodal elongation may be genetic mutation or environmentally induced. Hyperelongation in rosette plants is brought about byl the application of small amounts of gibberellins (Fig. 8.16).

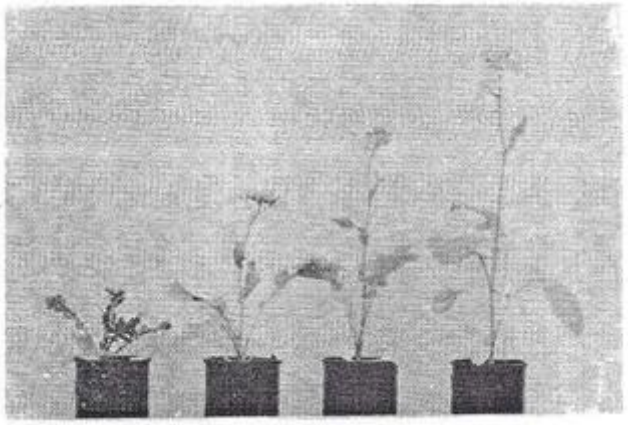

Fig. 8.16 Gibberellin - stimulated stem growth in a rosette genotype of *Brassica napus*

Environmentally limited rosette plants (Spinach, Cabbage) do not flower in the rosette form. Just before flowering these plants will undergo extensive internode elongation, known as bolting. Bolting is triggered either by photoperiodism or a combination of low temperature and photoperiod. Rosette plants can be induced to bolt by an exogenous application of GA.
Spinach contains six gibberellins, including GA_{19} and GA_{20}will cause bolting in spinach under short day conditions while GAl9 is biologically inactive. J.A.D. Zeevaart and coworkers found that rosette plants of spinach contain high levels of the inactive form GA_{19} and low levels of the active GA_{20}. Upon transfer to long day conditions, the level of GA_{19} declined while the level of GA_{20} increased (Fig. 8.17). It may be concluded that GAs has a significant role in the control of stem elongation in rosette plants.

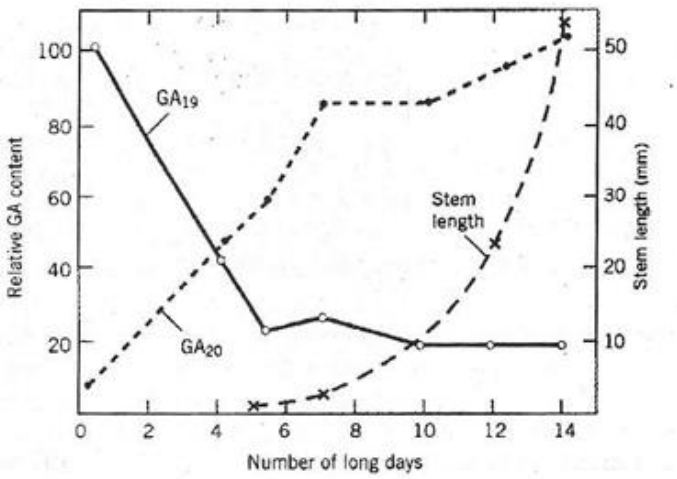

Fig. 8.17 Changes in gibberellin content accompanied by the transfer of spinach (*Spinacea oleraceae*) plants from short to long days exhibit extensive stem elongation.

The relationship between gibberellins and stem elongation in cold- requiring plants has not been studied as thoroughly as it has for photoperiodically sensitive plants. Exogenous application of GA3 will substitute for the cold requirement in many plants and there is some evidence that gibberellin-like activity increases in plants following cold treatment.

Inhibition of stem growth: The growth of many stems can be reduced or inhibited by synthetic chemicals that block GA biosynthesis. These growth retardants or antigibberellins mimic the dwarfing genes by blocking specific steps in GA biosynthesis, thus reducing endogenous GA levels and suppressing internode elongation (Fig. 8.18).

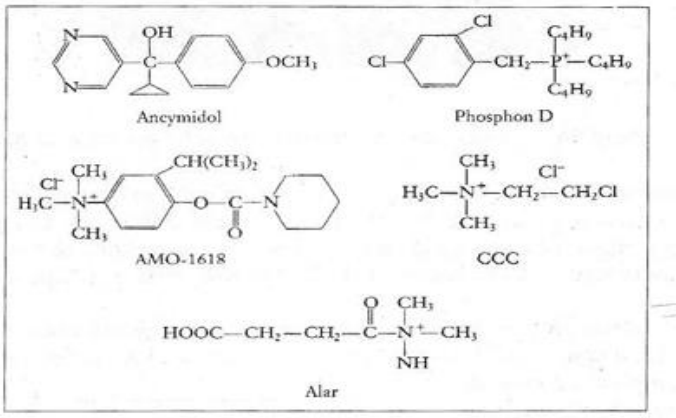

Fig. 8.18 Chemical structures of growth retardants

In some areas, wheat tends to "lodge" near harvest time, that is, it becomes top-heavy within grain and falls over. Spraying the plants with growth retardants produces a shorter, stiffer stem and thus prevents lodging. Growth retardants also need for pruning of vegetation. Alar has been widely used as a spray on cherries and apple. It enhances fruit colour and produces a firmer fruit that facilitates harvesting.

Seed germination: Germinating cereal grains secrete ex-amylase and proteases which digest carbohydrates and protein. Cereal grains produce two half-seeds, with one half-seed containing the embryo and one without. The embryo-containing half-seed will proceed to secrete ȁ-amylase, digest the starchy endosperm and germinate. The embryo less half-seed cannot germinate and does not produce elevated levels of ex-amylase or any other hydrolytic enzymes required for germination. GA treatment will stimulate the embryo less half-seed to produce high levels of ȁ-amylase' (Fig. 8.19). GA stimulated ȁ-amylase secretion can be blocked by inhibitors such as actinomycin D and cycloheximide, which inhibit RNA and protein synthesis. This indicates that gibberellin-stimulated de novo synthesis of a-amylase by the aleurone layer is an early event in germination. seeds grow at high temperatures, produce high levels of ȁ-amylase in the absence of added GA.

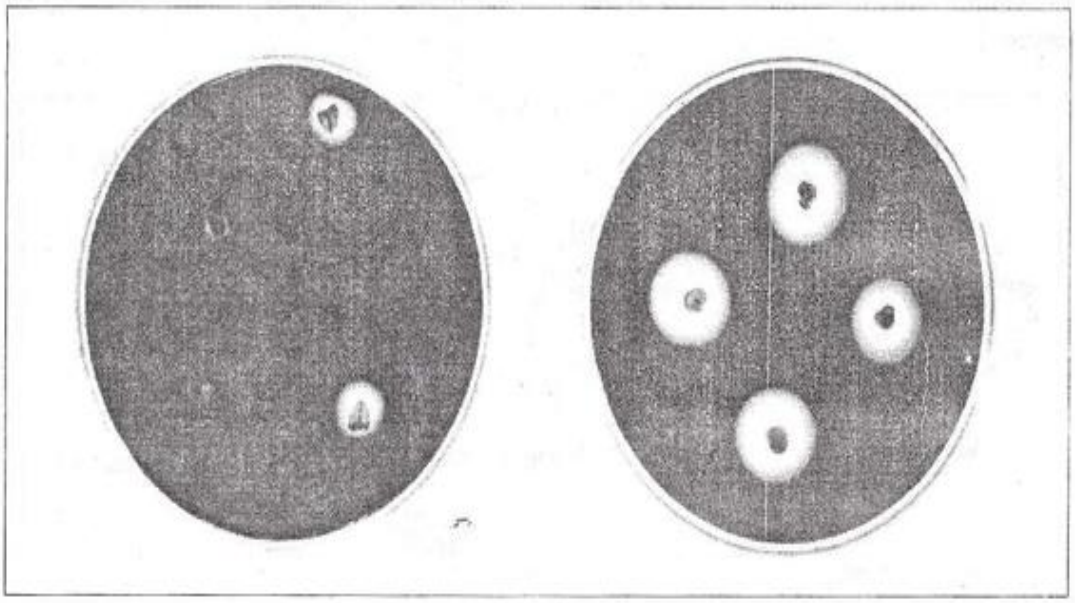

Fig. 8.19 Gibberellin - stimulated release of a-amylase from barley half seeds

Flowering: In the normal course of development, stem elongation appears to be a necessary prelude to flowering. Substitution of gibberellin for the long day or cold requirement seemed to indicate a role for gibberellin in the flowering process itself. Inhibitors of GA biosynthesis will suppress photoperiodic - induced stem elongation without interfering with flowering. It appears that stem elongation and flowering are separate. Though gibberellins do not play a role in flowering, they do influence the capacity of plants to flower as well as sexual characteristics of flowers and fruit development.

Many perennial plants must achieve a minimum stage of development before they can flower. Such plants are said to pass through a juvenile phase. The length of the juvenile phase can range from a few weeks to many years. In cases like Cucumis and Cannabis sativa, where auxins or ethylene promote femaleness in imperfect flowers, an application of gibberellins will promote formation of male flowers.

8.4 CYTOKININS

Cytokinins are N6-substltuted derivatives of the nitrogenous purine base adenine, characterized by their ability to· stimulate cell diving in tissue culture. Kinetin (N6- furfuryl amino purine) was the first cytokinin to be discovered (Fig. 8.20). Kinetin does not occur naturally but was synthesized from herring sperm DNA.

The most widespread naturally occurring cytokinin in higher plants is Zeatin. Zeatin is round with a ribose (the riboside) or ribose-phosphate (the riboside) at the 9-position. In addition to stimulate cell division, cytokinins also influence shoot and root differentiation in tissue culture, the growth of lateral buds and leaf expansion, chloroplast development and leaf senescence.

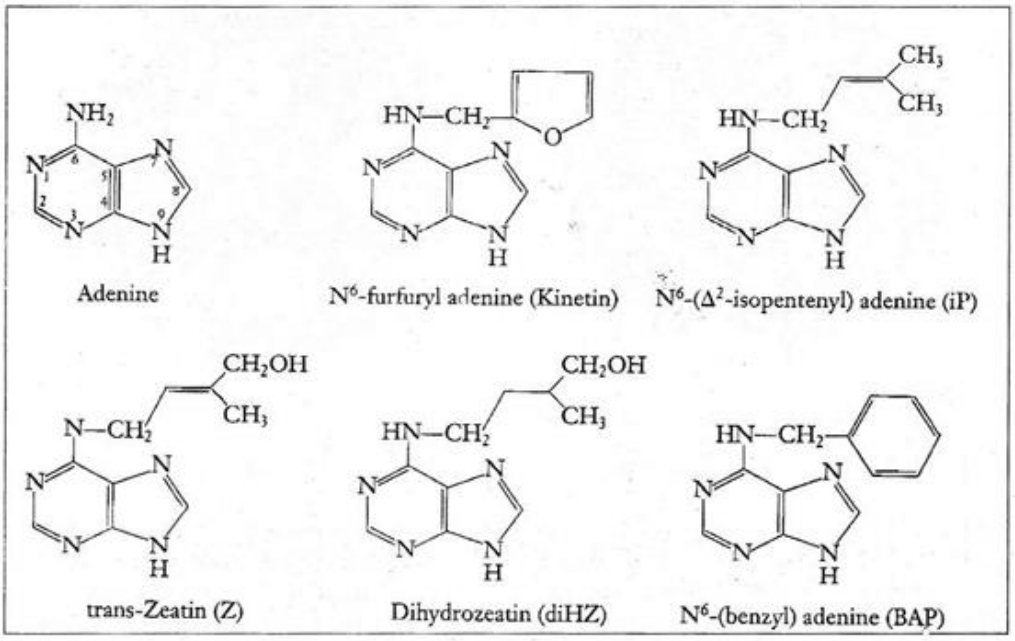

Fig. 8.20 The chemical structures of adenine and five adenine derivatives with cytokinin activity.

8.4.1 Cytokinin Biosynthesis and-metabolism

A major site-of cytokinin biosynthesis and high cytokinin levels in higher plants are the mitotically active root tip, in the xylem sap of root and in immature seeds and developing fruits.

8.4.2 Biosynthesis of cytokinins

Cytokinins are commonly found in the cell as modified bases in transfer RNA (tRNA) and methylated purines. These cytokinins are not incorporated during transcription of the tRNA but are synthesized during post-transcriptional processing.

Enzymes direct de novo synthesis of cytokinins from adenosine-5' monophosphate have been isolated from the slime mold *Dictyostelium discoideum* tobacco callus tissue, and crown gall tissue (Fig. 8.21). This reaction is specific for the nucleotide; the enzyme will not add the isoperitenyl group to either adenine or adenosine. The product. [9R-5'P] iP, is the precursor to

all other naturally occurring cytokinins. Little [9R-5'PJ iP accumulates in tissue and undergoes a rapid hydroxylation of the side chain to give the comparable zeatin ribonucleotide. Reduction of the double bond in the side chain would give the dihydrozeatin derivative, while sequential hydrolysis of the phosphate group and the ribose moiety would give rise to zeatin.

Cytokinins undergo interconversion between the free base, ribosides and the ribosides when supplemented to tissues. Enzymes have been identified in wheatgerm that catalyze the conversion of iP to its riboside (9R) iP or to its ribotide ([9R-5'P] iP) as well.as enzymes that catalyze the hydrolysis of the ribotides and ribosides to the free base (iP).

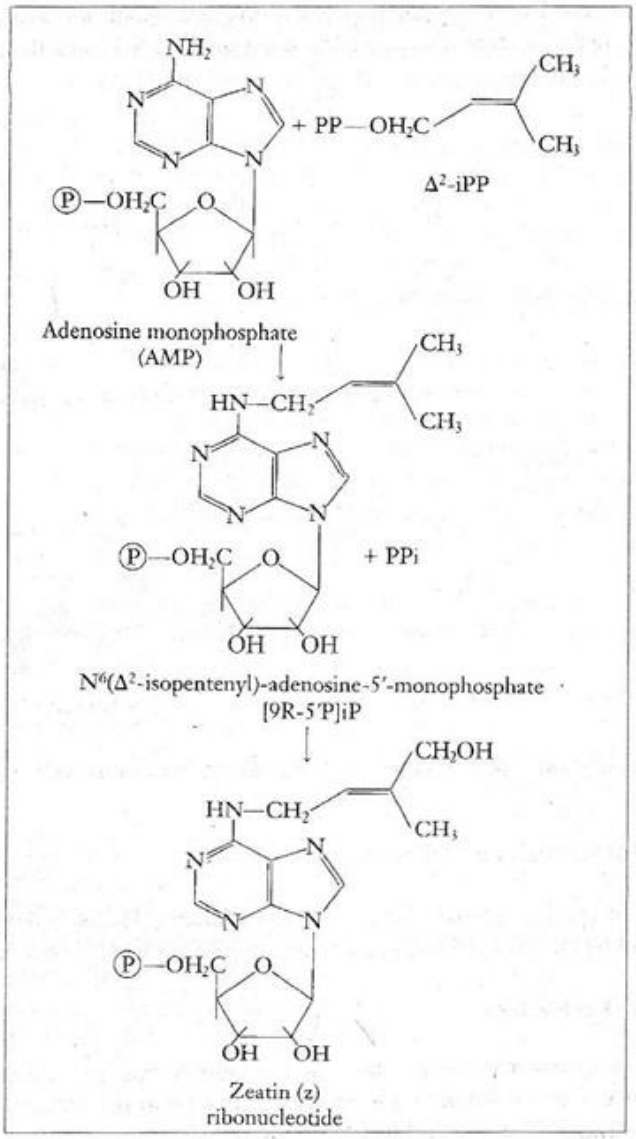

Fig. 8.21 The pathway for *de novo* cytokinin biosynthesis

8.4.3 Cytokinin metabolism and transport

Conjugation with either glucose or amino acids and oxidation are two principle routes for regulating cytokinin activity. Glucose conjugates are formed at the nitrogen in position 7 or in 9 on the purine ring or as O-glucosides on the sidechain (Fig. 8.22). The 7- and 9-

glucosides are biologically inactive, and O-glucosides are biologically very active. The N-glucosyl conjugates are very stable and do not appear to be hydrolyzed to give the active gee base. O-glucosides appear to be storage forms that are readily hydrolyzed to yield biologically active cytokinins when needed by the plant.

Cytokinins form conjugates with the amino acid alanine (Fig. 8.23). 9-Alanyl conjugates of zeatin and dihydrozeatin have been identified. These too are very stable conjugates that serve to inactivate the cytokinin in the same manner as N-glucosides. A major route for removal of exogenously supplied cytokinins in many tissues is oxidation by the enzyme cytokinin oxidase. Cytokinin oxidase, partially purified from tobacco tissue, maize and crown gall tissue, cleaves the isopentenyl side chain from either Zeatin or iP or their ribosyl derivatives (Fig. 8.23).

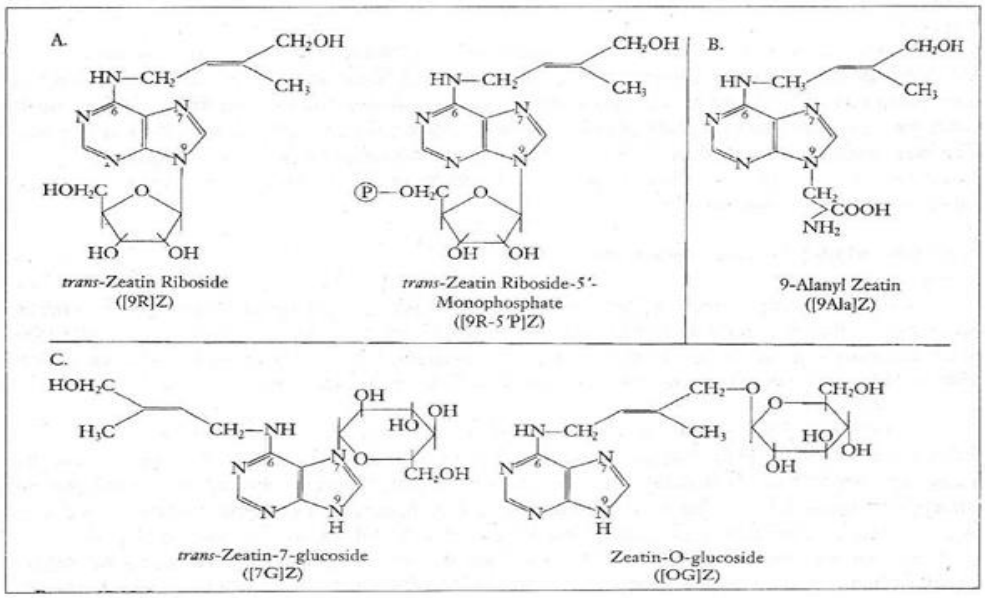

Fig. 8.22 Examples of Zeatin conjugates

8.4.4 The Physiological roles of cytokinins

Cell division and morphogenesis: Most mature, differentiated plant cells do not normally divide. Many cells may be induced to undergo division when cultured in artificial media containing vitamins, mineral salts, a carbon source and an optimal concentration of hormones. On solid agar medium, cells derived from stem pith and cortex, cotyledon, leaf, and other tissues. will divide and enlarge to produce a mass of undifferentiated cells referred to as Callus tissue. Small lumps will also form in liquid culture on agitation and form a cell free culture. In both cases, cell division and growth do not occur in the absence of cytokinin.

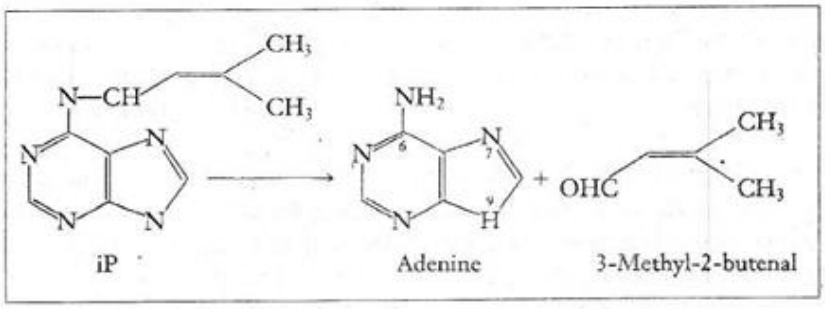

Fig. 8.23 Oxidative degradation of iso-pentenyl adenine (iP) by cytokinin oxidase

Cytokinins also influence morphogenesis in cultured tissues. High molar ratio of cytokinin to auxin tends to induce bud development, and high ratios of auxin to cytokinin will encourage root development. It is possible by manipulating cytokinin and auxin balance in the medium, to regenerate complete plantlets from undifferentiated callus tissue in sterile culture. The plantlets can be transplanted into soil in the green house or field where they grow into fully competent, mature plants. This capacity to regulate morphogenesis in cultured tissues has referred to as micropropagation.

Nutrient mobilization and senescence

When a mature leaf is detached from a plant, it undergoes a process known as senescence. Senescence is characterized by the breakdown of protein, nucleic acid and other macromolecules, a loss of chlorophyll and the accumulation of soluble nitrogen products such as amino acids. Senescence is a normal consequence of the aging process.

There are three kinds of evidence indicating a role for cytokinins in control of senescence. First is that exogenous application of cytokinin to detached leaves and. to the leaves on intact plants will delay the onset of senescence, maintain protein levels, and prevent chlorophyll breakdown. The second evidence is that detached leaves have been treated with auxin.to induce root formation at the base of the petiole will remain healthy for weeks. The growing root is a site of cytokinin synthesis, and the hormone is transported through the xylem to the leaf blade, If the roots are continually removed as they form senescence of the leaf will be accelerated. When a mature plant begins its natural senescence, there is. a sharp decrease in the level of cytokinins exported from the root. Third, evidence comes from recent studies employing recombinant DNA techniques. Tobacco plants have been transformed into the *Agrobacterium* gene for cytokinin biosynthesis, designated *tmr.* The *tmr* gene encodes for the enzyme iso-pentenyl-transferase. The tmr gene was linked to a heat shock promoter. The heat shock promoter is active only when subjected to a high temperature treatment. By linking the tmr gene to the heat shock promoter, cytokinin biosynthesis can be turned on in the transformed plants simply by subjecting the plants to a brief period at high temperature.

There is evidence that cytokinins exert a role in mobilizing nutrients (Fig. 8.24). In K Mothes and coworkers' experiment, a nutrient labelled with radioactive carbon ^{14}C-glycine) is applied to a leaf after a portion of the leaf has been treated with cytokinin, the radioactivity is transported to and accumulated in the region of cytokinin treatment. It is unlikely that cytokinins act directly through stimulating protein synthesis since the mobilization of non-metabolites such as a-aminoisobutyric acid is directed by cytokinins equally well.

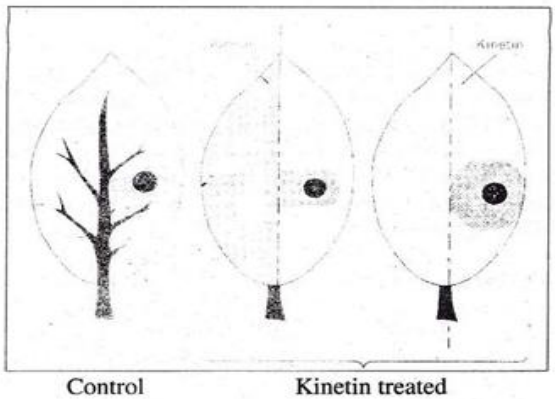

Fig. 8.24 Experiment demonstrating the role of cytokinin in nutrient mobilization.

Other cytokinin effects: Cytokinin will stimulate cell enlargement in the cotyledons of cucumber and sunflower. The application of cytokinins will stimulate release of axillary buds from apical dominance, thus antagonizing the effect of auxins. This cytokinin– auxin antagonism is believed to account for the phenomenon of "Witch's broom", an example of extreme axillary bud release (Fig. 8.25). It is believed that parasitism by fungi and bacteria stimulates an over production of cytokinin. The resulting release of apical dominance produces a dense mass of short branches.

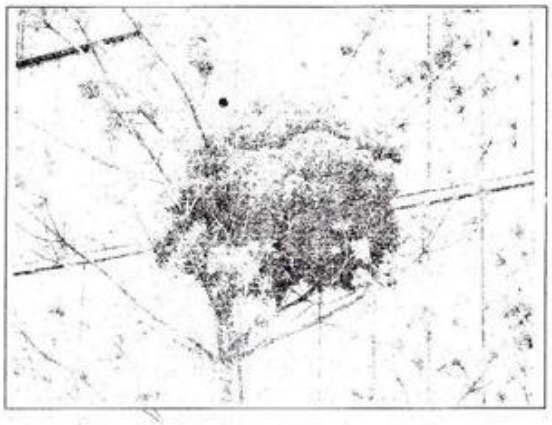

Fig. 8.25 Witch's broom on white pine (Pinus strobus)

8.4.5 Mechanism of cytokinin action

The action of cytokinin is poorly understood. There is some evidence that cytokinins have a role in regulating protein synthesis. In cultured soybean cells, cytokinins cause an increase in the overall rate of protein synthesis and change the pattern of proteins that incorporate 35S-methionine. This activity is reflected in an increase in the polyribosome content of cultured cells following cytokinin treatment. An increase in polyribosomes might result from either an increase in the rate of transcription of mRNA or an increase in the stability of the mRNA. Either brief irradiation with low level red light or the addition of cytokinin to the medium will stimulate an increase in the abundance mRNA for both the small subunit of ribulose-I, 5-bis phosphate carboxylase-oxygenase and the principal chlorophyll a/b-binding polypeptide of the light-harvesting complex. E. Tobir and her colleagues were able to demonstrate that transcription of the mRNA is under red light while cytokinin seems to stimulate an increase in the abundance of mRNA. A reasonable interpretation of these results is that cytokinin acts post-transcriptionally to stabilize the mRNA.

8.5 ABSCISIC ACID

Unlike the previous three hormone classes, abscisic acid (ABA) is a single compound (Fig. 8.26). Two major areas of ABA action appear to be in the mobilization of reserves during seed development and germination and in the response to leaves to water stress. ABA is known to induce transport of photosynthate toward developing seeds and to promote the synthesis of storage protein. During germination, ABA antagonizes the promotory effect of gibberellin H C on ȁ-amylase synthesis. Relatively large amounts of ABA are rapidly synthesized in the leaves in response to water stress, where it appears to play a major role in regulating stomatal opening and closing.

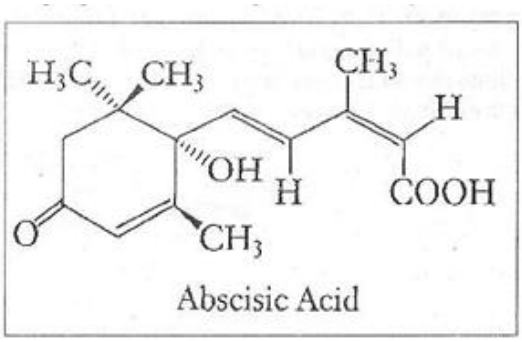

Fig. 8.26 The chemical structure of abscisic acid

8.5.1 Abscisic acid biosynthesis and metabolism

Abscisic acid is a 15-carbon isoprene derivative that appears to be synthesized by cleavage from a 40-carbon carotenoid precursor. ABA appears to occur in nature, green leaves and is synthesized in the chloroplasts, Stress induced ABA synthesis occurs in the chloroplasts and that the ABA migrates to other regions of the plant. Chloroplasts should probably be considered a major site of ABA synthesis.

ABA is a 15-carbon sesquiterpene and is also derived from mevalonic acid. Like other hormones ABA is present in very low concentrations, that is, 10 to 50 ngm/g fresh weight. Only in water-stressed leaves, where the concentration may reach 400 ngm/g fresh weight, or in young developing seeds do ABA concentrations exceed these values.

There are two pathways for the ABA synthesis: (1) direct synthesis from a 15-carbon precursor, or (2) cleavage of a 40-carbon xanthophyll (Fig. 8.27). The intermediate for direct synthesis of ABA is the 15-carbon farnesylpyrophosphate.

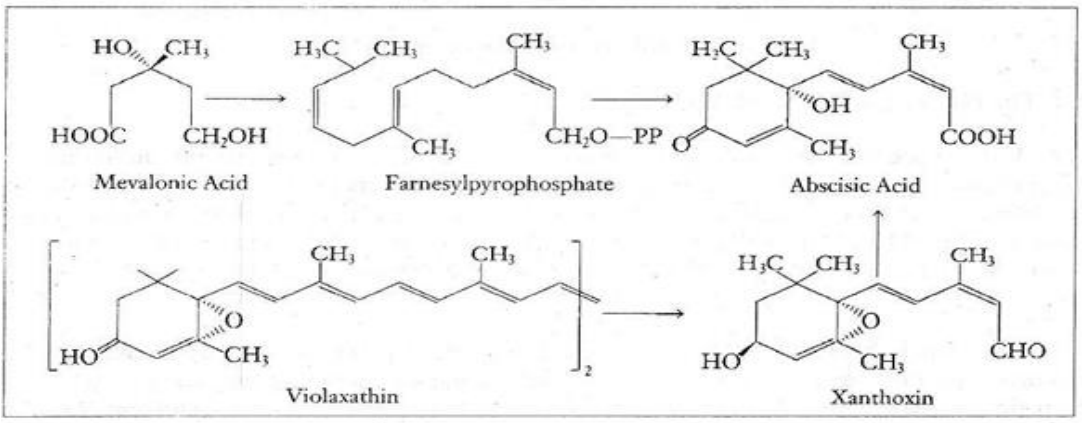

Fig. 15,27 Pathways for the biosynthesis of abscisic acid

First, the carbon skeleton of ABA and the position of the oxygen- containing substituents is very similar to that of violaxanthin. Second, it is known that violaxanthin can be degraded in the light in vitro to a IS-carbon derivative, xanthoxin, a natural constituent of plants. Third, a stoichiometric relationship between losses of violaxanthin and increases in ABA in stressed etiolated bean leaves.

ABA contains an asymmetric carbon atom. An asymmetric carbon atom gives rise to two different forms called enantiomers. There are two enantiomeric forms of ABA, designated R-

ABA and S-ABA. ABA is rapidly metabolized when it is applied exogenously to plant tissues. A glucose ester of ABA has been found in low concentration in a variety of plants, but the principal metabolic route seems to be oxidation to phaseic acid (PA) and next reduction of the ketone group on the ring to form dihydrophaseic acid (DPA) (Fig. 8.29). At least some tissues appear to carry the metabolism further to form the 4' -glucoside of DPA. DPA and its glucoside are both metabolically inactive. PA, while inactive, is equally effective as a GA3 antagonist in barley aleurone-ex-amylase system.

Like gibberellins, ABA is found in both xylem and phloem fluids as well as in parenchyma cells outside the vascular tissue, and there is no evidence for polarity in transport.

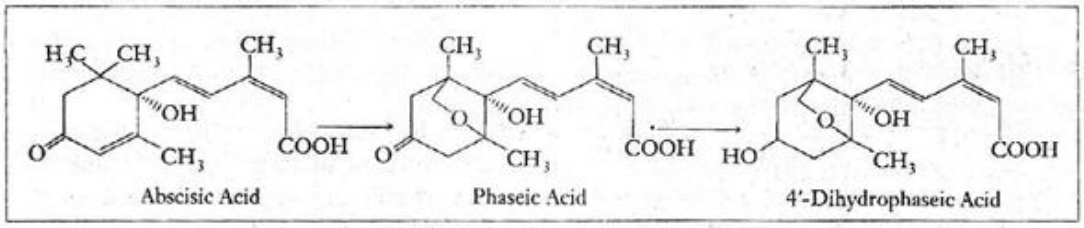

Fig. 8.28 Oxidative degradation of ABA

8.5.2 The Physiological roles of ABA

Most temperate zone woody plants experience a cessation of shoot growth during the growing season. This applies to both terminal and axillary buds. Shoot growth ceases normally in the late' summer or early fall, terminal and axillary buds enter a period of physiological dormancy. The intensity of dormancy reaches a maximum in early to mid-winter and then gradually declines, in response to chilling temperatures, until normal bud regrowth occurs in the spring.

It seems that ABA plays an important regulatory role in two processes, seed maturation and stomatal function. In most seeds, cytokinin levels are highest during the very early stages of embryo development when the rate of cell division is also highest. As the cytokinin level declines and the seed enters a period of rapid growth, both GA and lAA levels increase. In the early stages of embryogenesis, there is little or no detectable ABA. In the later stages of embryo development, as GA and IAA levels begin to decline, ABA levels begin to rise. ABA levels peak during the maturation stage when seed volume and dry weight also reach a maximum. Maturation of the embryo is characterized by cessation of seed growth, accumulation of nutrient, reserves and the development of tolerance to desiccation.

ABA serves to prevent **vivipary**, precocious germination before the embryo reaches maturity or the seed is released from the fruit. Precocious germination will occur when the ABA concentration is reduced to 3 to 4 μg/g fresh weight of seed. A number of viviparous mutants in *Arabidopsis* and corn have reduced level of ABA. Vivipary can also be induced in corn by treatment of the developing ear at the appropriate time with fluoridone, a chemical inhibitor of carotenoids. Carotenoids and ABA share early biosynthetic steps, fluridone inhibits ABA biosynthesis. Fluoridone induced vivipary can be alleviated by application of exogenous ABA. All of these results establish a strong connection between ABA and seed maturation and/or prevention of precocious germination.

In leaves of plants that have been grown to ensure minimum endogenous levels of ABA, exogenous ABA at concentrations of 10^{-3} to 10^{-4} M will induce complete stomatal closure.

This appears to be a means for regulating water balance in the plant since the endogenous level of ABA in leaves is generally very low if the plants are well watered. Subjecting leaves to water deficit. will induce as much as forty-fold increase in the ABA level within as little as 30 minutes.

8.5.3 Mechanism of ABA action

Based on in vivo labeling experiments and cell-free translation of barley aleurone mRNA it is clear that ABA suppresses GA-induced synthesis of ά-amylase and other hydrolases. it also promotes the synthesis of several ABA-specific polypeptide. The effect of ABA can be overcome by providing an excess of GA. ABA controls a-amylase at more than one level. Several studies have shown that ABA operates at the transcriptional level to suppress accumulation of GA-induced a-amylase mRNA, but an ABA-induced inhibitor of a-amylase activity has also been identified in mature starchy endosperm. Thus, it appears that ABA can prevent germination not only by suppressing transcription of a-amylase but also by inhibiting the activity of any enzyme that might be present in the endosperm.

8.6. ETHYLENE

Ethylene is a simple gaseous hydrocarbon with the chemical structure: $H_2C = CH_2$. Ethylene is not required for normal vegetative growth, although it can have a significant impact on the development of roots and shoots.

Ethylene is synthesized in response to stress and produced in large amounts by tissues undergoing senescence or ripening: Ethylene commonly used to enhance ripening in bananas and other fruits that are picked green for shipment. Ethylene is frequently produced when high concentrations of auxins are supplied to plant tissues. Many of the inhibitory responses to exogenously applied auxin appear to be due to auxin-stimulated ethylene release than auxin itself.

8.6.1 Ethylene biosynthesis and metabolism

Ethylene occurs in roots, sterns, leaves, bulbs, tubers, fruits, seeds and so on. Ethylene production will vary from tissue to tissue within the organ but is located in peripheral tissues. M. Lieberman and L.W. Mapson (1964) first showed that. methionine was converted to ethylene in a cell-free, non-enzymatic model system: These confirmed that plant tissues such as. apple fruit, converted L-[^{14}C]-methionine to [^{14}C)-ethylene and ethylene was derived from the third and fourth carbons of methionine. In 1977, when D. Adams and F. Yang demonstrated that S-adenosyl methionine (SAM) was an intermediate in the conversion of methionine to ethylene by apple tissue. In 1979, they further demonstrated the accumulation; of 1-.aminocyclopropane-l-catboxylic acid (ACC), a non-protein amino acid, in apple tissue. fed L-[^{13}C]-methionine under anaerobic conditions.

There are three steps involved in ethylene biosynthesis (Fig.8.29). In the first step, an adenosine group is donated to methionine in the presence of methionine adenosyl transferase by a molecule of ATP, thus forming SAM. In the presence of ACC synthase, SAM is cleared to yield 5' -methylthio-adenosine (MTA) and ACC. The Ethylene-forming enzyme catalyzes the conversion of ACC to ethylene. This enzyme system has been studied in cells, protoplasts and intact vacuoles. Ethylene production is promoted by IAA, wounding, and water stress. Induction of ACC synthase is blocked by inhibitors of both protein and RNA synthesis. This

suggests that induction occurs at the transcriptional level. In E. coli carrying the cloned ACC synthase gene, the physical abundance of ACC synthase mRNA also increases in response to IAA and wounding. Control of ethylene production appears to be exercised through transcriptional regulation of the ACC synthase gene.

8.6.2 The physiological roles of Ethylene

Ethylene is known to affect plant growth and development. As a byproduct of hydrocarbon consumption, ethylene is also a common environmental pollutant that can play havoc with greenhouse cultures or laboratory experiments. The gas chromatograph has made possible quantitative analysis of ethylene at extremely low concentrations that could not otherwise be measured. Etheption (2-chloroethyl phosphonic acid) is a compound that readily decomposes to produce ethylene, at physiological pH.

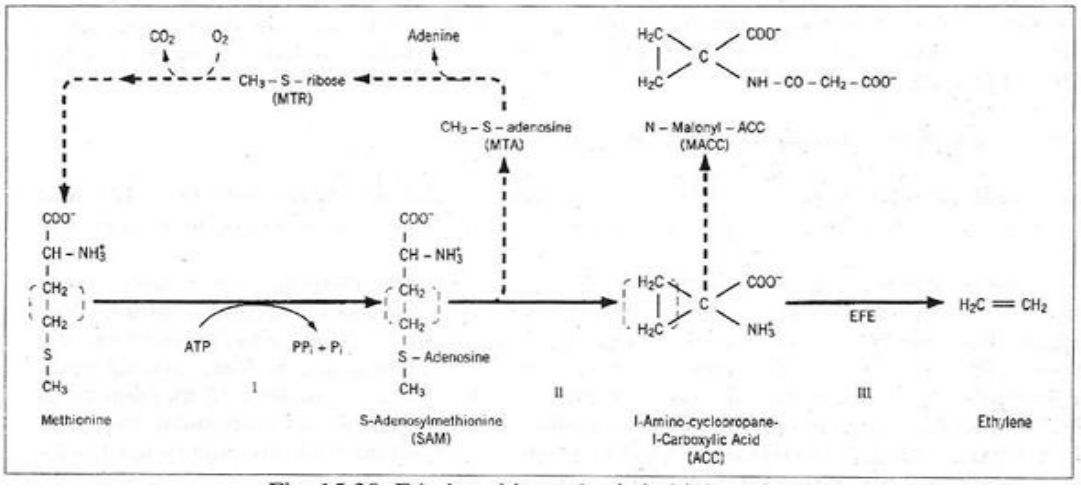

Fig. 8.29 Ethylene biosynthesis in higher plants

Vegetative development: Ethylene has been shown to stimulate elongation of stems, petioles, roots and floral structures of aquatic and semiaquatic plants. The effect is particularly noted in aquatic plants because submergence reduces gas dispersion and maintains higher internal ethylene levels. Ethylene promotes gibberellin synthesis in rice, and the elongation effect can be blocked with antigibberellins, which suggests that gibberellin mediates the ethylene effect. Ethylene stimulates abnormal growth responses such as swelling of stem tissues and the downward curvature of leaves; promotion of seed germination, inhibition of bud break, reduced apical dominance and root initiation.

Fruit development: A variety of fruits release ethylene gas during the latter stages of the ripening process. The release is coincidence with a sharp rise in the respiratory rate measured by CO_2 evolution. Ethylene is autocatalytic, i.e., ethylene released by ripening fruits will in turn stimulate premature climacteric and ethylene production by other fruits stored nearby. As a consequence of the climacteric and ethylene production, a number of qualitative metabolic changes such as hydrolysis of starches to sugars, softening of the tissue through the action of cell wall degrading enzymes and synthesis of pigments and flavor components. The nonclimacteric fruits such as citrus will show enhanced ripening when exposed to ethylene gas. Ethylene stimulated ripening is of considerable economic importance. For long-term storage, apples are placed under conditions designed to minimize the production and accumulation of ethylene. These include low temperature and high ambient CO_2 concentration to suppress respiration or continuous exchange of air to prevent a buildup of ethylene.

Flowering: Ethylene normally suppresses or delays flowering. Ethylene stimulates flowering in the family Bromeliaceous, eg,, pineapple. This is known to be due to auxin-stimulated ethylene generation. Commercial growers now use ethephon and other ethylene releasing agents to stimulate uniform flowering in pineapple fields.

8.7 SUMMARY

Hormones are naturally occurring organic substances. At low concentrations they effect on the function and development of organisms.Auxins were the first plant hormones to be discovered. These are synthesized in the stem and root apices and transported through the plant' axis. They are characterized by their capacity to stimulate cell elongation inexcisedstern and coleoptile sections.irootinijiation,vascular differentiation, tropic responses and the development of axillary buds, flowers and fruits. " Gibberellins are produced- by both' fungi arid higher plants. The exogenous application of gibberellin causes hyperelongation of' Intact 'stems; involved in seed gerininatibn 'and mobilization of endosperm reserves during early embryo growth and flower and fruit development. Cytokinins. are, 'N6-substituted derivatives, of, the nitrogenous .purine . base adenine,"characterized by their, abUhy to stimulate cell division Abscisic acid is a terpenoid derivative involved in regulating seed germination, inducing storage protein synthesis and modulating water stress. Ethylene is a gaseous hydrocarbon. It is not required for normal vegetative growth but it can have a significant impact on the development of roots and shoots. Its synthesis is stimulated by auxin. Ethylene is noted for its role in stimulating fruit development.

8.8 MODEL QUESTIONS

1. What are plant hormones? Explain about the physiological action of auxin.
2. Give a brief note on gibberellin biosynthesis, metabolism and transport.
3. Physiological functions played by gibberellin.
4. What are cytokinins and discuss briefly about the physiological roles played by cytokinins?
5. Discuss briefly about the biosynthesis and metabolism of auxins.
6. Explain about biosynthesis, metabolism and transport of cytokinins.
7. Write an essay on biosynthesis, metabolism, transport and physiological roles played by Abscisic acid.
8. Biosynthetic pathways, metabolism and physiological roles played by ripening hormone.

8.9 REFERENCE BOOKS

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Dr. Madhuri vajha

LESSON -9

THE FLOWERING PROCESS- PHOTOPERIODISM AND ITS SIGNIFICANCE, VERNALIZATION, ENDOGENOUS CLOCK AND ITS REGULATION

OBJECTIVES :

In this lesson, you will learn:

1) Juvenility: the early phase of growth during which flowering cannot be induced by any treatment. Physiological and morphological changes during juvenile phase.
2) Photoperiodism: including the distinction between short day plants, long day plants other response types, the central role of the dark period, the nature of photoperiodic perception and a discussion of the elusive floral hormone.
3) Timing of biological processes by the internal biological clock.
4) Time measurement in photoperiodism, including the hour-glass hypothesis and the role of the biological clock.
5) Vernalization - the low temperature requirement for flowering 10 winter annual and biennial plants.
6) The significance of photoperiodism in nature.

CONTENTS

9.1 INTRODUCTION

The development of most plants is seasonal with developmental processes and flowering occur at a specific time of the year. They are immediate responses to environmental conditions like temperature, water supply or light intensity. In fact, seasonal changes in plants are rarely controlled in this way but depend on certain characteristics of the environment. It may be difficult for a layman to believe that plants can tell time. One example is the consistent flowering of various species at particular times of the year. Roses always bloom in the summer and chrysanthemums in the fall. In the northern latitudes, perennial plants sense the short days of autumn as a signal to induce bud dormancy, thus anticipating the unfavorable conditions of winter.

Photoperiodism and vernalization are two major mechanisms underlying such seasonal responses and the words indicate the characteristics of the inductive environmental signals: Photoperiodism combines the Greek words for light and length of time. It is the length of day that gives the most reliable indication of the advancing season and an organism's capacity to measure day length is known as photoperiodism. Flowering is either quantitatively or qualitatively depended on exposure to. low temperature. This phenomenon is known as vernalization.

Photoperiodism is a response to the length of day and vernalization is an effect on flowering brought about by exposure to cold. These two mechanisms and their combinations enable plants to make seasonal adaptations.

Vernalization plays an additional role in seasonal flowering and found in regions with winter temperatures that are unfavorable for growth. A requirement for exposure to cold for several weeks will prevent flowering until winter has been experienced by the plant. Germination in late summer would not lead to immediate flowering in favorable photoperiods and vernalization becomes a device to ensure that the winter has passed before the onset of reproduction.

Juvenility, vernalization and photoperiodism are three important processes that determine when plants flower with respect to both ontogeny and season.

9.2 JUVENILITY

Juvenility is the name given to the early phase of growth during which flowering cannot be induced by any treatment. The juvenile duration varies widely. In woody plants, it lasts for several years (30-40 years), in herbaceous plants it is quite short, more than few days or weeks in duration. In extreme cases, there is no juvenile phase since flower primordia are found in the seed. At the time of transition from juvenile to mature, physiological and morphological changes

occur like leaf shape, thickness, leaf retention, thorniness, phyllotaxis, pigment content, rooting capacity etc. (Table 9.1).

Table 9.1 Juvenile and adult characters of English; Jy (Hedera helix L.)

Juvenile character	Adult character
Three-or five-lobed palmate leaves	Entire, ovate leaves
Alternate phyllotaxy	Spiral
Anthocyanin pigmentation of young leaves and stem	No anthocyanin pigmentation
Stems pubescent	Stems glabrous
Climbing and plagiotropic growth habit	Orthotropic growth habit
Shoots show unlimited growth and lack terminal buds	Shoots show limited growth terminated by buds with scales
Absence of flowering	Presence of flowers

The transition from juvenile to adult appears to be associated with changes in the apex, the base of the plant may remain in the juvenile condition even after the transition to maturity has occurred at the apical meristem.

The attainment of maturity:
The transition to maturity is not necessarily accompanied by flowering. Even a fully mature tree may not flower if it is growing vigorously and irregular flowering is common in many trees. Lack of flowering does not necessarily indicate the juvenile condition.

It is agreed that size is important, it is not yet clear what component of size is critical for attaining maturity. Two views have been examined. One is that a plant of sufficient size transmits one or more signals to the apex. The second is that the apical meristem behaves independently and undergoes the phase transition at a particular time.

Nutritional and hormonal factors:
The apex receives both nutrients and hormones from the rest of the plant. Factors like low light and high temperature reduce the supply of carbohydrate to the apex. This causes rejuvenation or prolong the juvenile phase in many plants. It is evident that, endogenous gibberellins may play a role in phase transition. Although many factors cause reversion to the juvenile condition, only time and / or size can bring about the transition to maturity. The presence of roots close to the apex has been suggested to be important in maintaining juvenility. In ivy (*Hedera helix)*, the aerial adventitious roots produced at the nodes of juvenile plants have a high concentration of extractable gibberellins and removing the roots decreased the number of GA-like substances in the shoot apices.

9.3 PHOTOPERIODISM

In 1912, a French scientist J.Tournois found that both *Humulus* (hops) and *Cannabis* (hemp) plants flowered precociously during the winter in the greenhouse. Tournois eliminated temperature, humidity and light intensity. In 1914, he concluded that the shortening of day length or lengthening of night was responsible for early flowering. H. Klebs observed that, *Sempervivum funkii* grown as a vegetative rosette in the winter green house. By supplementing normal daylight with artificial light, Klebs was able to stimulate stem elongation and induce flowering. From these experiments, Klebs concluded that length of day triggered flowering in nature. W.W. Garner and H.A. Allard demonstrated the full impact of daylight on flowering and coin the term Photoperiodism.

Garner & Allard conducted experiments with a mutant cultivar of tobacco (*Nicotiana tobacum)* called Mary land Mammoth. In the field, these plants grew to be very tall with large leaves. During normal growing season, these plants would not flower. In green house, even very small plants flowered in the winter and early spring.

On soybeans (*Glycine max*). When the cultivar Biloxi was sown over a three-month period from May to August, all the plants flowered within a three-week period in September. It appeared that all plants, regardless of age, were simply awaiting some signal to initiate flowering.

Like Tournois, Garner and Allard eliminated environmental conditions and finally concluded that flowering was controlled by the length of day and night. They went on to suggest that bird migration might also be keyed to day length. We now know that photoperiodic control is not limited to flowering but is a basic regulatory component in many aspects of plant and animal behavior.

Photoperiodic response types:

Photoperiodic responses fall into three categories. They are short-day plants (SD plants), long-day plants (LD plants) and day-neutral plants (DNP).
Short day plants: - *Chenopodium rubrum*
Chrysanthemum sp.
Cosmos sulpliureus
Euphorbia pulcherrima
Xanthium strumarium
Long day plants:- *Beta vulgaris*
Raphanus sativus
Triticum aestivum
Dry neutral plants:- *Cucumis sativus*
Helianthus annum
Phaseolus vulgaris

Short-day plants are those that flower earlier in response to day lengths that are shorter than a value within a 24-hour cycle. Long-day plants respond to day lengths that are longer than a value, and day-neutral plants flower irrespective of day length. In addition to these three basic categories, there are various species of the genus *Bryophyllum* are example of long-short-day plants (LSD plant). They will flower only if a certain number of short days are preceded by a certain number of long days. *Trifolium repens* is an example of short-long-day plant (SLD plant).

Intermediate - day length plants flower only in response to day lengths of intermediate length but remain vegetative when the day is either too long or too short. Another type of behaviour is amphophotoperiodism, flowering is delayed under intermediate day length (12 to 14 hours) but occurs rapidly under day lengths of 8 hours or 18 hours.

Photoperiodic induction

Many plants require continuous exposure to the appropriate photoperiod in order to flower successfully. Others will proceed to flower even' if, the plant is returned to unfavourable

photoperiods. Such plants are called induced, and the appropriate photoperiod is called an inductive treatment.

9.3.1 The central role of the dark period

Garner & Allard suggested that plants responded to the relative lengths of day and night. The term photoperiodism implies that plants measure the duration of day light. In fact, plants measure neither the relative length of day and night nor the length of the photoperiod. Instead, they measure the length of the dark period.

This was demonstrated by the experiments of K'C. Hamner and J. Bonner in 1938 under 24~hour cycles of light and dark, Xanthium flowered with dark periods longer than 8.5 hours but remained vegetative on schedules of 16 hours light and 8 hours dark (Fig. 9.1 A-H). The numbers in brackets indicate the length of dark period. Note that the plants flower whenever the dark period is uninterrupted for nine hours or more.

On schedules of 4 hours light-S hours darkness, plants remained vegetative even though the 4-hour photoperiod is much shorter than the 8.5 - hour critical photoperiod (Fig. 9.lC). Schedules of 16 hours light - 32 hours darkness induced rapid flowering even though the photoperiod exceeded the critical daylength (Fig. 9.1D). The above results showed two conclusions:

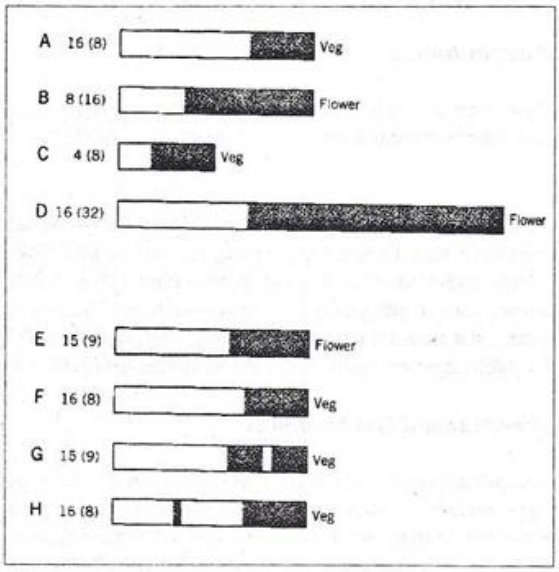


Fig. 9.1 The central role of dark period in *Xanthium strumarium*, a SD plant

(1) the relative length of day and night is not the determining factor in photoperiodism.
(2) it is the length of the dark period that is important.

The consistent feature of these experiments is that Xanthium will flower whenever the dark period exceeds 8.5 hours and will remain vegetative whenever the dark period is less than 8.5 hours.The flowering effect of an inductive 9-hour dark period can be nullified by interrupting the dark period with a brief light-break (Fig. 9.1G), but a "dark interruption" of a long light period has no effect (Fig. 9.1H). At this point, photoperiodism has little to do with daylength, rather it is a response to the duration and timing of light and dark periods.

9.3.2 The role of Phytochrome

If the leaf perceives the photoperiodic stimulus, then the leaf must be capable of measuring time. How leaves measure time is not clear, but we do know that phytochrome is involved. Early action spectra on several SD plants and LD plants in the late 1940s indicated that red light was most effective as a light-break, with a maximum near 660 nm. H.A. Borthwick and his colleagues reported that red, far-red photoreversibility of the light-break clearly implicates phytochrome in the photoperiodic timing process. The role of phytochrome is far from clear at this point but based on recent work it is suggested that PHY A is required to promote flowering of an LDP under certain conditions. PHY B seems to inhibit flowering.

9.3.3 Light requirements and floral hormones:

The Russian plant physiologist M. Chailakhyan in 1936 suggested that the floral stimulus might be a hormone. He proposed the name f1origen. Chailakhyan and many investigators have shown that floral stimulus can be transmitted through a graft union. When several Xanthium plants are approach-grafted in sequence, all can be brought to flower if only the first is induced by short days (Fig. 9.2).

In some of the early experiments, it was suggested that florigen, like auxin, could be transmitted through a non-living connection. Experiments involving anatomical studies proved that transmission of the stimulus occurred only when a tissue union had been established.

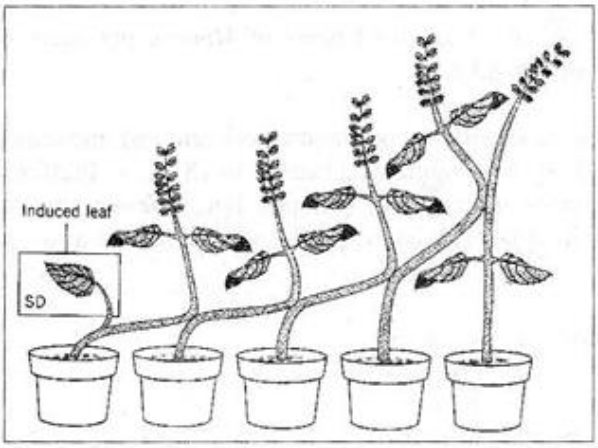


Fig. 9.2 Transmission of the floral stimulus in grafted plants

Auxins, cytokinins and ethylene have been reported to either enhance or suppress flowering in various species. Of these hormones, only gibberellins evoke flowering in a wide variety of species. Lang (1957) showed that repeated applications of dilute gibberellin solutions to the apex of annual LD plants elicited a flowering response under short days. But florigen and gibberellins are not equal.

Chailakhyan proposed that flowering is under control of two hormones. Gibberellin and a hypothetical substance, anthesin. Since gibberellin does not promote flowering in SD plants, he proposes that SD plants have sufficient gibberellin but lack anthesin. Synthesis of anthesin is stimulated by short days. LD plants, would have sufficient anthesin but lack adequate levels of gibberellin. Gibberellin synthesis is stimulated by long days. Day-neutral plants would tlower irrespective of day length because they are able to synthesize adequate levels of both the "hormones".

9.3.4 Temperature and Photoperiodism

In some cases, the flowering response is simply enhanced at certain temperatures while others respond at high or low temperatures. Winter cereals will not normally flower during a single growing season but must be planted in the fall in order to flower and produce a crop the following year. Spring strains will flower and produce grain in the same year they are planted.

9.3.5 The Biological Clock

French astronomer M. De Mairan in 1729 raised that nyctinastic movements are due to exogenous control namely, the daily pattern of light and dark periods and internal or endogenous timekeeper might be involved. Leaf movements of Mimosa persisted even when the plants were placed in darkness for several days. In 1863, J. Sachs reported no correlation between leaf movements and temperature fluctuations, thus eliminating temperature as a cause. In 1875, W. Pfeffer devised an apparatus for automatic and continuous recording of leaf position. Pfeffer attached a leaf, via a fine thread, to a stylus, which in turn recorded the position of the leaf on a rotating drum coated with carbon (Fig. 9.3).

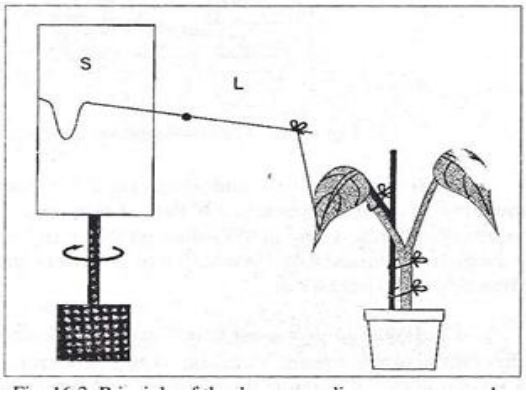
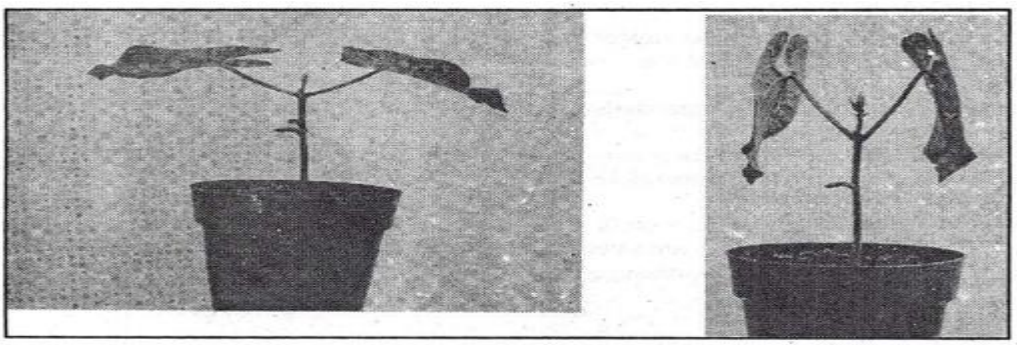


Fig. 9.3 Principle of the drum recording apparatus used by Bunning and others for recording of leaf movements.

Pfeffer contributed several papers devoted to leaf movements in Phaseolus vulgaris (Fig. 9.4). At one point, he showed that plants that had lost their rhythmic leaf movements will regain them if exposed to a new light-dark cycle. If the new cycle is inverted with respect to natural day and night, leaf movement will also be reversed. He concluded that persistent leaf movements under continuous light or darkness were a "learned" behaviour. In the end, Pfeffer was forced to conclude that leaf movements were an endogenous probably inherited behavior.



A graphic plot of a biological rhythm against time describes a repeating pattern that resembles physical wave phenomena. Much of the terminology describes physical oscillations has been adopted to describe biological cycles. At the beginning, it is necessary to distinguish between simple periodic phenomena and endogenous rhythms. For example, photosynthetic carbon uptake describes periodicity because it is light-driven and daylight is periodic over time. Photosynthesis is diurnal, in that it is active only during day light hours and is controlled by fluctuations in an external factor (light). The key to an endogenous rhythm is that it persists for several cycles, under constant conditions (constant light and constant darkness). The rhythmicity expressed under constant conditions is called free-running.

The time required to complete a cycle is known as the period (T, tau) (Fig. 9.5A, B).Period is conveniently described as the time from peak to peak, but it applies equally well to any two comparable points in the repeating cycle. Constant rhythms are classified according to the length of their free-running period. Thus a circadian rhythm has a period of approximately 24 hours. Rhythms in metabolic activity with periods less than 24 hours (measured in minutes or hours). These are known as ultradian rhythms.

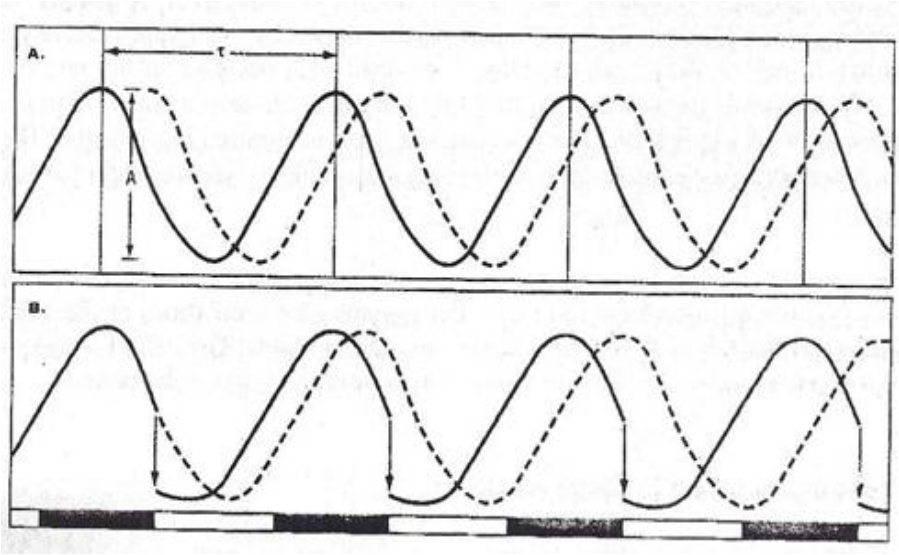


Fig. 9.5 Examples of circadian oscillators

Discussions of endogenous circadian rhythms are complicated by the fact that two time frames involve solar time and circadian time. Solar time based on a normal 24 hour day, circadian time is based on the free-running period. One cycle is considered to be 24 hours long, regardless of its actual length in solar time. Each hour of circadian time is therefore 1/z4 of the free-running period: Thus, if the free-running period is 30 hours, events that occur at 0, 15 and 30 hours of darkness will have occurred at circadian time, s CT : 0, CT : 12 and CT : 24 (Table 9.2).

The phase of the free-running cycle that corresponds today in a normal' light-dark environment is known as subjective day and that which corresponds. to normal night is the subjective night.

Table 9.2. Example of circadian rhythmic phenomena in higher plants

Rhythm	Organism
Sleep movements	Many speci[illegible]
Stomatal opening	Banana, tobacco, *Vicia*
Stem growth	Tomato
CO_2 production	Orchid flowers
Gas uptake	Dry onion seeds
Membrane potential	Spinach leaves
mRNA expression	Pea

Most chemical reactions and growth and other biological responses respond to temperature (with a QIO near 2) a ro-c increase in temperature will approximately double the rate of the process. A decrease in temperature leads to a decrease in the rate by the same amount. When seedlings are raised in the dark from seed, leaf movements tend to be small and unsynchronized. A single flash of light initiates larger synchronized movements. The circadian rhythm is temperature-sensitive, but some mechanism quickly compensates for variations in temperature. The action of the biological clock or endogenous rhythms is to ensure that certain functions occur at a particular time of day. For example, the oscillations of the clock in beans determines that the leaves rise during the day and fall at night. The period of the endogenous rhythm is fixed but it may be "fast" or "slow" relative to the 24-hour solar period.

9.3.6 The measurement in photoperiodism

With the discovery that flowering in SD plants was determined by the length of a critical dark period, it seemed that the length of the critical dark period could be measured simply by the appearance and disappearance of some metabolites. Such an approach to timing was likened to an hourglass (Fig. 9.6) which, when the sand runs out, must .ie inverted to restart the timing I process. This hypothesis is called "hourglass" hypothesis.

Fig. 9.6 An hourglass timer

When the action spectra showed that phytochrome was the photoreceptor responsible for light break phenomenon. It is concluded that Pfr, present at the end of photoperiod was inhibitory to flowering: The length of the critical dark period represented the time required for Pfr to fall below some critical value long enough to allow for synthesis of florigen. The effectiveness of

a red light break in the middle of an inductive long dark period was seen simply as raising the level of Pfr, thus restarting the timing process before sufficient florigen could.be produced.

In 1936, Buning proposed that the rhythm was comprised of two phases, the photophile or light loving phase and the scotophile, the dark-loving phase, which altered about every 12 hours. According to Bunning's hypothesis, light falling on the plant during the photophile phase would promote flowering and light during the scotophile phase would inhibit flowering. When the plant is placed under continuous conditions, the photophile phase is equivalent.to subjective, day and the scotophile phase is equivalent to subjective night.

9.3.7 Genetic approach

If a mutant can be identified that influences timing at any level, the wild type gene can be isolated and its gene product analyzed for clues to its role in the timing mechanism. Flowering genes have been a part of plant breeding programmes for years. Because early flowering and insensitivity to photoperiod are most desired in crop species. Two crop species peas, (*Pisum sativum)* and wheat (*Triticum aestivum*) have the genetics of photoperiodic processes received much attention. Both the crops are quantitative long-day plants. In peas, several genes that affect photoperiodic timing and the onset of flowering have been identified. **fsd** (flowering short days) is a recessive mutant that causes the plant to behave as a qualitative short-day plant. When the mutant is grafted to a wild type stock under long days, the mutant will flower.

Arabidopsis is a quantitative long-day plant with a photoperiod of 8 to 10 hours. Under long! days, it flowers with 4 to 7 leaves in the rosette (about 3 weeks), and during short days, flowering is delayed until 20 leaves (7 to 10 weeks). Flowering is also promoted by exposure to blue or far-red light. This reflects the role of phytochrome in photoperiodic phenomena. Here, flowering time refers not to the elapsed time (days to flowering) but to the number of rosette leaves 'produced before the flowering stem appears. Mutants that affect phytochrome also influence flowering. The hy l mutant is defective in the synthesis of the phytochrome chromophore. In the absence of functional photoreceptor, hy l mutants show an elongated hypocotyl, flower earlier than wild type under both long and short days. Because they flower early under both conditions, the mutant shows a response to photoperiod.

There are certain other mutants like early flowering (elf 3) is an example of early flowering type and Constans (co) and gigantea (gi) are late flowering, which are day length insensitive mutants. Both co and gi delay flowering under long days but no effect on flowering time under short days. When wild type *CO & GI* are studied, the Gl gene operates before the CO gene in the same pathway and that floral promotion under long days depends on the amount of CO mRNA transcribed. The 'elf 3 mutants, interacts with endogenous clock, elf3, also effects leaf-movement and transcription of the chlorophyll a/b binding protein (CAB). The rhythm: for CAB expression is not lost if the entrained plants are shifted to continuous dark. This indicates that ELF3, does not encode a component of the endogenous clock itself, but links the clock to the initial photoreception or light-on signal,

9.3.8 Photoperiodism in Nature

Photoperiodism reflects the need for plants to synchronize their life cycles to the time of year. Photoperiodism is more important to plants in the subtropical and temperate latitude where

seasonal variations in day length are more pronounced. Many tropical plants respond to the small changes in day length that occur within 5 or 10 degrees of equator. This does not mean that the photoperiod response ties to a species to a particular latitude, since the critical photoperiod sets the upper (for SO plant) or lower (for LO plant) limits of daylength.

Photoperiodism helps to ensure that plants flower in their temporal niche. This helps the plants to reduce competition with others and ensures that the reproductive development is completed before the onslaught of unfavourable winter conditions. If flowering relied solely on plant size, non-uniform germination would be expected 'to spread flowering out in time. In cross-pollinating plants, flowering synchronized by photoperiod would' serve to ensure maximum pollinating population or coordinate with the appearance of particular' pollinating insects.

9.3.9 Influence of Temperature on Development

The temperature in tropical climates is relatively stable. Plants growing in temperate regions and closer to the poles show variations in temperature on a daily and seasonal basis. ' Plants have evolved ways to incorporate this information in their developmental and survival strategies. Plants use this information to ensure dormancy of -buds, tubers and seeds and to modify their flowering behaviour. These are keys for survival over the conditions unfavourable to normal growth and development.

The temperature at which biological processes can occur is limited by the freezing point of water on the low side and the irreversible denaturation of proteins on the high side. The temperatures between these two extremes are called cardinal temperatures. Living organisms are broadly classified according to their ability to withstand temperature. The plants that grow optimally. at low temperature (0°C to l0°C) are called psychrophiles, e.g. algae, fungi and bacteria. The plants which grow optimally between ,10ºC to 30°C are mesophiles. Thermophiles can grow between 30°C to 65°C.

The temperature for the growth of plants is 0°C to 45°C. Temperature compatibility is very dependent. As a rule, temperature optimum for growth reflects the geographical region' in which the species originated: The effects of temperature oil physiology and metabolism influence plant distribution called biogeography. Temperature related metabolic effects not only' limit di'strib~tio~but ha~e economic implications as well.

9.3.10 Temperature and flowering response

There is an interaction between temperature and photoperiod particularly with respect to flowering behaviour. In most cases, the' interaction results in changes in the length of the photoperiod or a tendency toward day-length' neutralityor an inability to flower ~hough at high or low temperature extremes. There are other plants in which flowering is either quantitatively or qualitatively dependent on exposure to low temperature. This phenomenon is known as vernalization. Low temperature treatments hasten flowering. Vernalization reflects the ability of a cold treatment to make a winter cereal behave like a spring cereal with respect to its flowering behavior.

9.4 OCCURRENCE OF VERNALIZATION

Vernalization occurs in winter annuals and biennials. Typical winter annuals are called "winter cereals" (wheat, barley & rye). "Spring" cereals planted in the spring come to flower and produce grains before the end of the growing season. Winter strains planted in the spring would fail to flower. Winter cereals are plated in the fall, they germinate and over winter as small seedlings, resume growth in the spring and are harvested about midsummer.

F.G. Gregory and O.N. Purns studied thoroughly vernalization on the Petkus strain of rye in 1930s. The spring strain is a quantitative, long-day plant. Under short days, flower initiation does not occur until after about 22 leaves have been produced requiring about 7.5 months. Under long days, flowering in the spring strain is initiated after about seven leaves have been produced requiring about two months. The winter strain is not an LDP. These plants germinate at normal temperature, flowers slowly, requiring 4, 5 months, regardless of day length. When the winter strains are planted in fall, receive an over winter low temperature treatment. When it resumes growth in the spring, the winter strain responds to photoperiod as the spring strain. Over wintering cold treatment can also be achieved by vernalizing the seed. This can be done by holding the germinated seed near 1°C for several weeks. This shows that the low temperature treatment does not alone promote early flower initiation, rather, the effect of vernalization is to render the seedling sensitive to photoperiod. Biennials are monocarpic plants that flower and die in the second season: Ex.:.*Beta vulgaris,* Cabbages' and related plants, carrots (Daucus carota), members of umbellifereae and some strains of black henbane. Biennials share 'with the winte'r annuals the property that subjecting the growing plant to acold treatment stimulates a photoperiodic response.

Biennials grow as a rosette, with short internodes in the first season (Fig. 9.7): During winter, the leaves die back, but the apical meristem remains protected. New growth in the following spring is characterized by stem elongation called' bolting, followed by flowering. The requirement for cold is qualitative/absolute. In the absence of cold treatment, biennials are maintained in the non-flowering rosette habit indefinitely. As a rule, winter-annuals biennials, vernalizable plants tend to respond as long-day flowering plants, though some biennials are day-indifferent following vernalization. Chrysanthemum varieties require vernalization before responding as a quantitative SDP. As a perennial, *Chrysanthemum* requires vernalization on an annual basis.

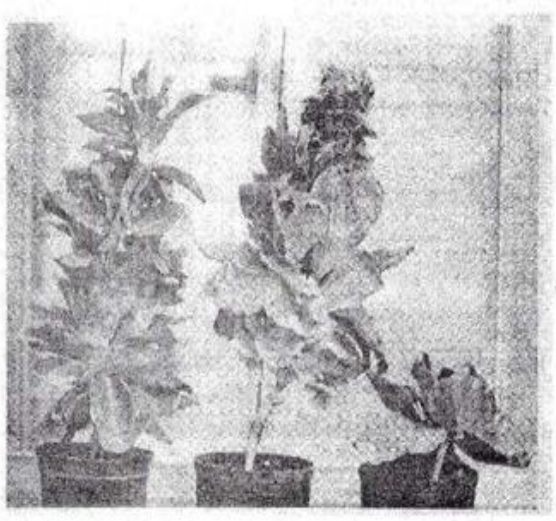


Fig. 9.7 Vernalization and stem elongation in cabbage (*Brassica sp*.)

9.4.1 Effective temperature

The range of temperatures varies depending on the species and duration of exposure. In Petkus rye, the effective range is - 5ºC to +15°C and optimum between $+1^0$C and $+7^0$C. Flowering advances after as little as one to two weeks treatment at 1°C to 2°C and is maximum effect after about seven weeks at that temperature (Fig. 9.8).

A longer exposure to lower temperatures within the effective range is required because the metabolic reactions leading to the vernalized state progress more slowly. This figure (Fig. 9.8) shows thai Petkus rye seeds were germinated in moist sand at 1°C for the time indicated. Cold treatments were scheduled so that all seeds were returned to the greenhouse at the same time. The number of days to flowering decreased with increasing length of the cold treatment. The vernalized state is permanent in most species giving rise to the concept of an induced state. All cold-requiring plants that have been studied are capable of being devenalized, a reverse state, if followed immediately by a high temperature treatment, Flowering in vernalized winter wheat can be nullified if the seedlings are held near 30°C for three to five days. For most plants there is a "neutral" temperature where neither vernalization nor devernalization occurs. For example. Petkus rye the neutral temperature is 15°C. Vernalized seeds can also be devernalized by drying them for several weeks or by maintaining the seeds under anaerobic conditions for a period following the cold treatment

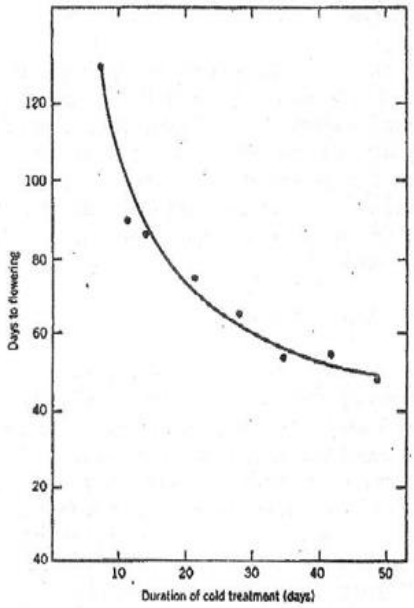


Fig. 9.8 Vernalization in Petkus rye (Secale cereale)

9.4.2 Perception of stimulus

A vernalization treatment is effective only on actively growing plants. Winter cereals may be vernalized as soon as the embryo has imbibed water, and the germination process has been initiated. The biennials must reach a certain maximum size before they can be vernalized. This can be shown by localized cooling treatments or vernalization of moistened embryos. Early studies by Gregory & Purys showed that even the cultured apex of isolated rye embryo was susceptible to vernalization. Thus, the induced state is established in a few meristematic cells that can be maintained through out the development of the plant. Most biennials car is not induced as seed. In these plants, it is t:le overwintering stem apex that perceives stimulus.

9.4.3 The nature of the vernalization stimulus

Vernalization is an energy-dependent metabolic process. Experiments with isolated embryos have shown that vernalization treatments are effective only when the embryo is supplied with carbohydrate and 02. A cold-induced, permanent change in the physiological or genetic state of the rneristematic cells would be self-propagating, that is. it could be passed on to daughter calls by cell division. In plants like Petkus rye and *Chrysanthemum,* only tissue produced in a direct cell line from the induced meristem is vernalized. If the cold treatment is localized to a single apex. it will flower. All the buds that did not receive the cold treatment will remain vegetative. In graft union experiments. the transmission of "florigen" across a graft union which results in flowering in non-vernalized receptor plants. If a vernalized *Hyoscyamus* plant is grafted to an unvernalized plant. both will flower under long days. Transmission requires a successful graft union together with the flow of photoassimilate between the donor and receptor.

G. Melchers proposed the existence of transmissible vernalization stimulus called Vernalin. Like florigen, vemalin has resisted ail aitemp'ts at isolation and remains a hypothetical substance. Adding to the complexity of vernalization is the apparent involvement of gibberellins in response to low temperature (Fig. 9.7). This was demonstrated by A. Lang (1957). He showed that repeated applications of 10 µg of GA_3 to the apex would stimulate flowering in nonvernalized plants of *Hyoscyamus* and other biennials and maintained under short days. It has been shown that gibberellin levels tend to' increase in response to low temperature treatments in several cold-required species. It is important to note that every situation in which gibberellin has substituted for long temperature or long days in promoting flowering involves bolting, the rapid elongation of stems from the rosette vegetative state. Less ~uccess has been achieved with gibberellins in caulescent LDP. - whose stems are already elongated in the vegetative state. Following low temperature treatment, flower buds are seen at the time stem elongation begins. Following gibberellin treatment, the stems first elongate to produce a vegetative shoot. Flower buds do not appear till later, M. Chailnkhyam suggested that vermalin is a gibberellin precursor. Vemalin would be accumulated in response to cold treatment in those plants requiring vernalization. But long days are required to complete its conversion to gibberellin.

9.5 SUMMARY

- Juvenility is the name given to the early phase of growth during which flowering can not be
- induced by any treatment.• All living organisms can be broadly classified according to their ability to withstand temperature. Psychrophiles grow optimally at temperature of O°C to $l0^0$C, rnesophiles at $l0^0$Cto 30°C and thermophiles at 30^0C to 65^0C. Most higher plants are mesophiles.
- Temperature is a principal factor in the distribution of plants. After experimentations, it is clear that temperature stability of principal metabolic pathway is a significant determinant in plant distribution.• Plants also us temperature as cue in their developmental and survival strategies.
- Vernalization is the promotion of flowering by a period of low temperature.• In case of winter annuals (cereals), vernalization changes the photoperiodic behaviour from daylength indifference to a quantitative long-day response. Biennials grow as rosettes until vernalized.

- The flowering stem then bolts and responds as a long-day plant.A temperature of 0^0C to 5^0C, applied to the actively growing apex of the plant for several weeks, is required for vernalization to be effective.

9.6 MODEL QUESTIONS

1. Write an essay on vernalization
2. Write short notes on the following:
   1) Juvenility
   2) The Biological Clock
   3) Genetic approach of photoperiodism
3. Give a brief account on photoperiodism

9.7 REFERENCE BOOKS

1) Introduction to Plant Physiology, 2nd edition, William G. Hopkins, John Wiley & Sons, Inc.
2) Advanced Plant Physiology, Malcolm B. Wilkins, English Language Book, Society/Longman.
3) Plant Physiology L. Taiz and E. Zeiger Sinauer Associates, Inc., Sunderland, Massachusetts.

Dr. Madhuri vajha

LESSON- 10

POLYAMINES AND BRASSINOSTEROIDS

OBJRCTIVE:

To learn about **Polyamines (PAs) and Brassinosteroids (BRs)** is to understand them as crucial plant growth regulators that enhance plant development, metabolism, and especially **stress tolerance (drought, salinity, heavy metals)** by interacting with other hormones, regulating genes, improving antioxidant systems, and affecting cell processes like elongation and division, offering significant potential for improving crop yield and resilience

STRUCTURE OF THE LESSON:

10.1 POLYAMINES AND BRASSNOSTEROIDS

POLYAMINES

The term polyamine refers to a group of polyvalent compounds containing two or more amine groups. They were first observed as crystals in human semen (hence, spermine) by van eeuwenhoek, with the help of his primitive microscope, more than 300 years ago. Polyamines began to attract the attention of plant physiologists in the early 1970s.Polyamines are derived biosynthetically from the amino acids arginine and lysine. In addition, spermidine and spermine biosynthesis involves S-adenosyl methionine, an intermediate in ethylne biosynthesis. In plant cells, polyamines occur conjugated with phenolic compounds such as hydroxy-cinnamic acid, coumaric acid or caffeic acid. At normal intracellular pH, polyamines are polycationic, that is, they carry multiple positive charges.Polyamines thus bind readily to nucleic acids, which are polyanionic and the phospholipids of the plasma membrane. It is possible that this binding character could effect the synthesis and/or activity of macromolecules and membrane permeability.Thermophilic bacteria produce polyamines as a means of, protecting against thermal inactivation of enzymes and sperminestabilizes DNA 'against therdtal 'de'mlruratiort in'vitro. Polyamines have been shown to be obligate growth factors for both prokaryote and eukaryote .microorganisms and mammalian cells and stabilize oat protoplastsvinfluericecell division and embryogenesis in carrot tissue-culture and delay

senescenceiin some tissues. Whether -polyamines can or should qualify as hormones may be an unprofitable effectsindicate a role in plants that clearly warrants further study.

Brassinosteroid

Hypothetical plant hormones: Studies' on flowering clearly indicate the transmission of a diffusible chemical signal from leaf to the apex. The existence-of a flowering hormone, called florigen and the hormone' vernalin have been postulated ~o account for the effect of low temperature on the flowering behaviour of winter cereals and biennials: "Florigen" might also be a complex' interaction between several regulatory molecules, rather than a single hormone. ' Other biologically active substances: The active substances, which are a complex mixture of lipids, known as brassins or brassinosteroids, have been isolated from pollen "Of the rape plant, Brassica napus L. This stimulated elongation of bean second internodes. One brassinosteroid is brassinolide (Fig. 10.2). Like auxins, brassinolide is active in micromolar concentrations arid stimulates elongation of hypocotyl and epicotyl tissue from a variety of legume seedling's ana coleoptide tissue from wheat.

Brassinosteroids or brassins are a recently discovered group of steroids that have distinct growth promoting activity in some plants especially in stems. These compounds were first isolated in 1979 from bee collected pollen grains of rape (Brassica napus), a mustard (hence the name brassins). Brassinosteroids are now known to be widely distributed throughout the plant kingdom. In chemical structure, brassinosteroids resemble steroid hormones of animals.More than 60 brassinosteroids have so far been identified from different parts of plants such as pollens, seeds, leaves, stems, roots and flowers. They cause marked biological effects on plant growth at very low concentrations (micro-molar concentrations). One of the very common, well known and biologically active brassinosteroids in plants is brassinolide whose structure is given in Fig. 10.3. Interestingly, it is chemically similar to insect moulting hormones, ecdysones.

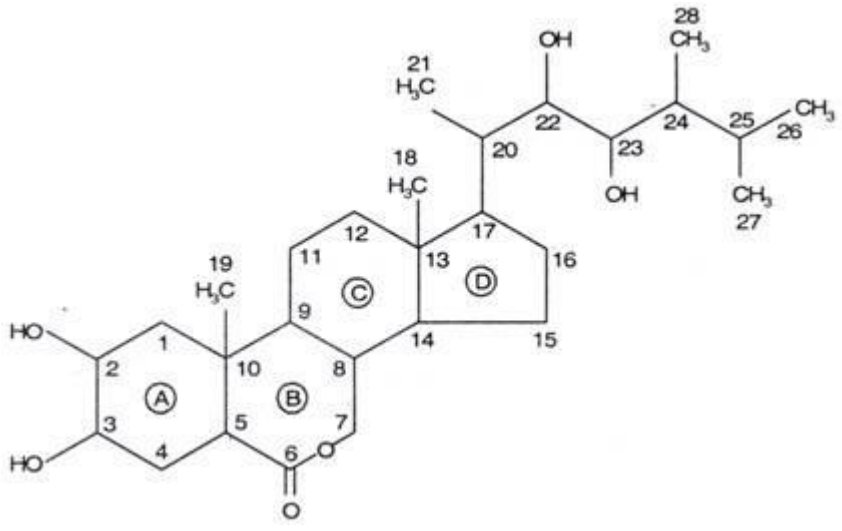


Fig10.2 Brassinolide

Physiological studies have established that exogenous brassinosteroid causes cell elon-gation and cell division in excised stem sections. Brassinosteroids also inhibit root growth, enhance gravitropism, promote xylem differentiation and growth of pollen tubes, stimulate seed germination and delay leaf abscission.

Because physiological effects of brassinosteroids qualitatively resemble to those of auxins, their role as plant hormones was not recognised earlier and it was believed that brassinosteroid acted partially by increasing sensitivity to auxins. Later on, evidences began to accumulate pointing towards plant hormonal nature of brassinosteroids.In soybean hypocotyls, brassinosteroids were found to affect cell elongation and gene expression quite independently of auxins. In auxin-insensitive mutant of Arabidopsis, root growth was found to be inhibited by brassinosteroids but remained uninhibited by auxins.

Conclusive evidences which led to wide acceptance of brassinosteroids as endogenous plant hormones came from discovery and analysis of two photomorphogenic mutants DET2 and CPt) of Arabidopsis in late 1990s by Chory et al at Salk Institute in Sant Diego. The genes DET2 and CPD encode enzymes which are involved in biosynthesis of brassinosteroids (Fig. 17.34). The light and dark grown phenotypes of DET2 and CPD mutants could be completely reversed to wild types by addition of brassinosteroid to the growth medium.Brassinosteroids also play important roles in many light and hormone regulated processes including the expression of light regulated genes, the promotion of cell elongation, leaf and chloroplast senescence and floral induction.

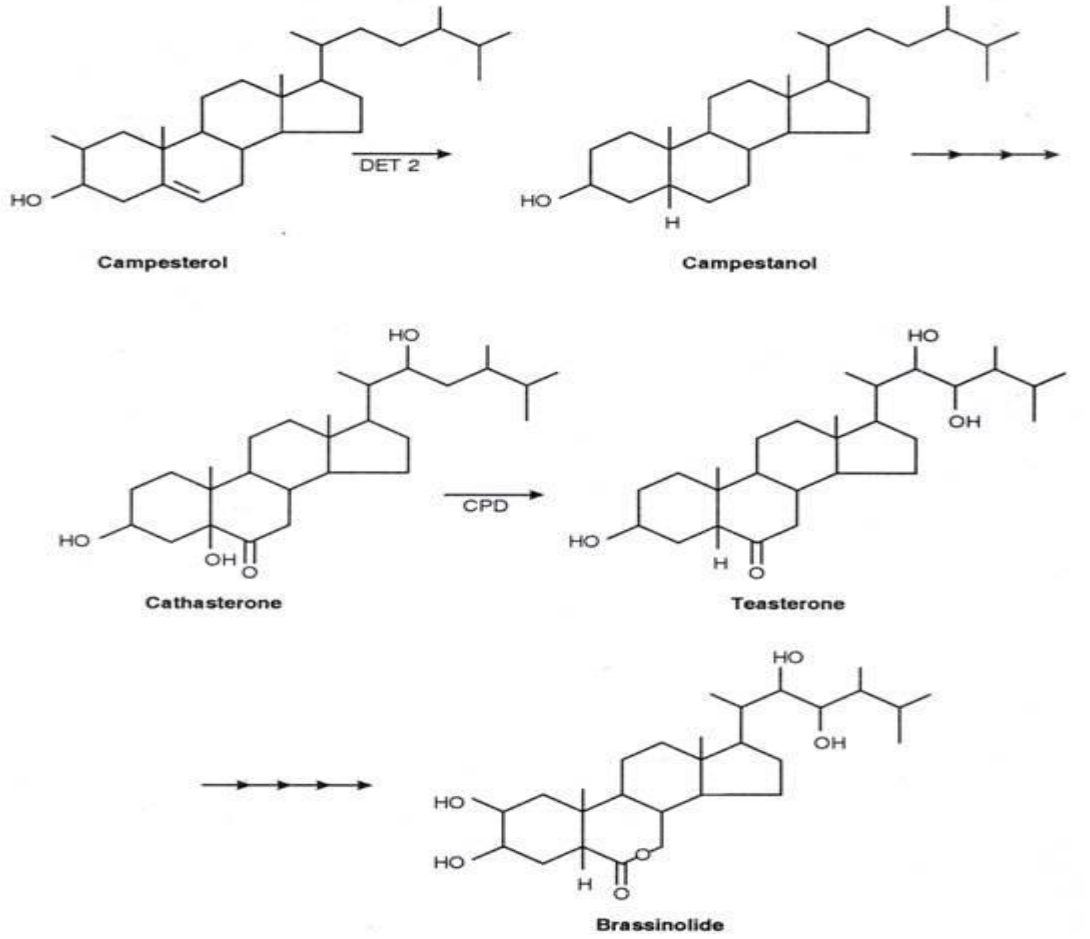


Fig 10.3 Biosynthetic Pathway for a brassinosteroide and brassinolide from campesterol

i. Brassinosteroids are biosynthesized from a C_{28} plant sterol called campesterol by a reductive step followed by several oxidative steps (Fig. 10.3). The biosynthesis of

brassinolide from campesterol requires app. 12 steps. The reductive step and one of the several oxidative steps are catalysed by enzymes DET2 and CPD respectively. Campesterol is in turn derived from a triterpene called squalene.

ii. Depending upon the species and the tissue involved, there may be several different routes for the deactivation of brassinosteroids in plants. There may be epimerization of α – hydroxyl groups to form β -hydroxyl groups followed by esterification with fatty acids or glucosylation. There may also be cleavage of side chain or conjugation at other hydroxyl situations.

iii. Brassinosteroids also have important agricultural implications in increasing productivity of many crop plants.

It operates synergistically with auxin toenhance the natural growth-promoting- activity of that hormone. Two other biologically active compounds of interest are Coumarin and transcinnamic acid. Coumarin is known to inhibit auxin-induced elongation of Avena coleoptiles and other tissues in vitro, .at low concentrations elongation may be promoted. Coumarin inhibits seed germination. Germination may proceed only after Coumarin levels in seed coat have been reduced by leaching. Trans-cinnamic acid also inhibits auxin activity in stem section assays and has been considered an antiauxin. The possibility that many of these biologically active substances may be used to increase yields has attracted attention in the fields of agriculture and horticulture.

10.2 BIOCHEMISTRY AND MODE OF ACTION OF HORMONES

The most challenging question about hormones is how· to hormones bring about profound effects on the physiology of cell? Despite years of research into hormone action and the effects of hormones on plants, our understanding of hormone action_at the molecular level is only just beginning. Experiments are thus designed to test whether those same principles might also apply to higher plants.

The sequence of events initiated by hormones can generally be resolved into three - sequential stages: (1) the initial signal perception, (2) a signal transduction pathway, and (3) the final response. 1) Signal perception involves the reaction of the hormone with a receptor site.

Plant hormones may diffuse from cell to cell either through plasmodesmata or through the apoplastic space. In either event, the cell destined to respond to the hormone, known as the target cell, must be capable of detecting the presence of the hormone molecule either in the cell or in the fluids immediately surrounding the cell. Detection is accomplished by interactions between the hormone and a cellular receptor that is both specific to the hormone molecule and characteristic of the target cell. Receptors are glycoproteins that bind reversibly with the hormone. As a result of binding the hormone, the receptor is induced to change its conformation and assumes an "activated" state. The formation of this active hormone-receptor complex completes the signal perception stage.

The second stage of hormone action is the signal transduction and amplification stage.The activated hormone-receptor complex sets into motion leads to the final, characteristic response.

Receptors for peptide hormones such as insulin and epinephrine are located on the extracellular surface of the plasma membrane (Fig. 8.32). The hormone receptor complex activates a membrane protein called the "G protein" which binds to a third membrane protein,adenylate cyclases - located at the cytoplasmic surface of the membrane. Binding of G protein to adenylate cyclase activate the enzyme. This stimulates the formation of cyclic adenosine monophosphate (cAMP) in the cytoplasm. The G protein may interact with an ion channel that controls the flow of calcium into the cell. The calcium will bind with one of a number of cytosolic calcium binding proteins, such as Calmodulin. The effect of either cAMP or the Ca+2 calmodulin complex is to activate specific protein kinases .

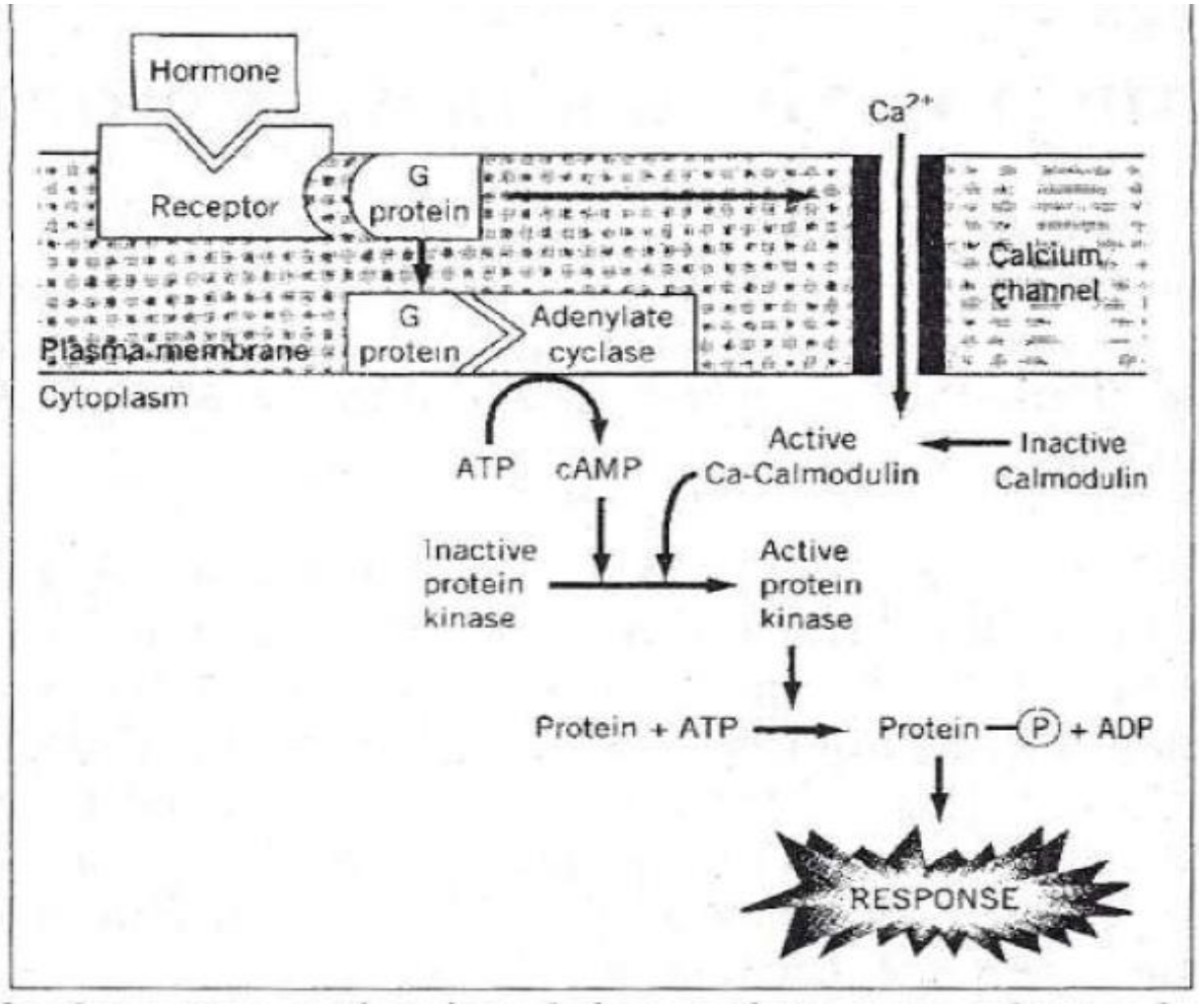


Fig. 10.4 A model for hormone action involving a plasma membrane-bound receptor

In this stage, the hormone is considered as first messenger, because it brings the original "message"to the cell surface. cAMP and calcium serve as second messengers. The function of second messenger is to relay information from the plasma membrane to the biochemical machinery inside the cell. Second messenger also provides for amplification of the-original signal.Steroid hormone receptors are located in the nucleus (Fig. 10.3). The hormone-receptor complex forms in the nucleus where it interacts with specific segments of DNA to either trigger or suppress transcription of mRNA.

Moreover, a finely tuned cellular regulation of BR levels is evident from the observation that increase in endogenous BR concentration lead to feedback regulation of the BR metabolic genes, while BR deficient conditions elicit the expression of BR biosynthesis genes to maintain BR homeostasis (Tanaka et al., 2005). Further to understand the BR mediated regulation of several key molecular and physiological functions in plants, extensive research have been conducted over past two decades (Zhu et al., 2013b). BR signaling involves its perception by the cell membrane receptor followed by activation of cascade of phosphorylation events (Figure 1) to relay the signal to the downstream partners resulting in the BR-induced gene expression (Belkhadir and Jaillais, 2015). The use of different biological approaches such as mutant screening, microarray, proteomics, protein–protein interaction studies and bioinformatics played vital role in identification and characterization of various components involved in BR signaling (Divi et al., 2010, 2015). Recent studies demonstrate that BR interacts at various level with the signaling components of other phytohormones and regulate process like plant growth and development and stress responses (Hu and Yu, 2014; Tong et al., 2014; Chaiwanon and Wang, 2015; Divi et al., 2015; Yuan et al., 2015). In the above background, the present review focuses on the recent advances in our understanding of the process of BR biosynthesis, transport, degradation and signaling. This information is useful in getting insights into dynamics of BR homeostasis and its implication in modulating various critical functions in plants. Present update also emphasizes the interaction between the key genes and transcription factors of BR with the signaling components of other phytohormones. This information will facilitate in getting insights into a fairly complex process of BR- mediated plant responses.

BRASSINOSTEROID HOMEOSTASIS AND ITS REGULATION

BR biosynthesis ensues from intricate network pathways and is mostly modulated by transcriptional regulation of BR biosynthetic genes (Chung and Choe, 2013; Vriet et al., 2013). Various genetic and biochemical studies have elucidated BR biosynthetic pathway which commences with campesterol, a precursor for synthesis of the most active form of BR, brassinolide(BL). Firstly campesterol is converted to campestenal which was initially believed to branch into two parallel pathways, namely the early and late C-6 oxidation pathways involving a chain of reductions, hydroxylations, epimerizations and oxidations which eventually converge at castesterone that leads to the formation of BL (Fujioka et al., 1998). Later studies revealed that BR biosynthetic pathway is a triterpenoid pathway (Choe, 2007; Chung and Choe, 2013). Mevalonic acid serves as a precursor of the triterpenoid pathway and is condensed and transformed to 2,3-oxidosqualene which further undergoes modification to form major plant sterols like sitosterols and campesterols.

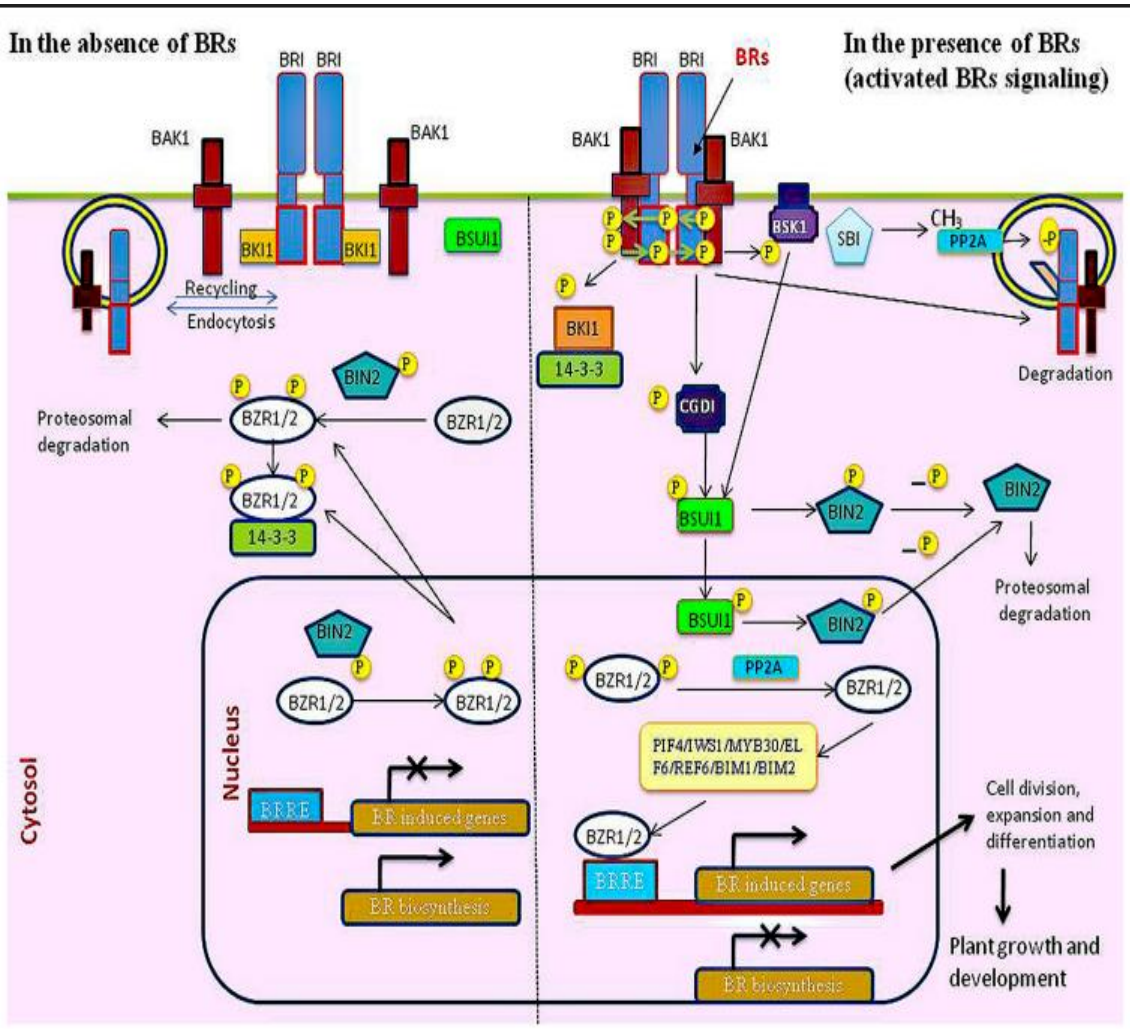


Fig. 10.5 A model for Brassinosteroid (BR) signaling pathway involving a nuclear hormone receptor

Hormone-binding proteins in plants Proteins bind readily to small molecules in a nonspecific manner, especially when tissues are disrupted during isolation protocols. There are four accepted criteria to distinguish between nonspecific binding and hormone-binding properties. First, binding must be specific. Second, the receptor should exhibit a high affinity for the hormone. Third, receptors can be saturated by increasing the concentration of hormone molecules. Fourth, the hormone must bind reversibly with the putative receptor.

10.3 BRASSINOSTEROID AS AUXIN BINDING PROTEIN

Auxin binding proteins (ABP) have been sought in two tissues, callus cultures of pith tissue (tobacco) and coleoptiles (maize). Three classes of IAA-binding proteins have been identified. Two are associated with membrane fractions (Plasma membrane) and one is found distributed between the cytoplasmic and nuclear fractions. One of the membrane-bound binding proteins has a low affinity for auxin but a high affinity for naphthylphthalamic acid (NPA), an auxin transport inhibitor. The second membrane-bound receptor has a moderate binding affinity for IAA but does not bind NPA.

The cytoplasmic-nuclear binding protein has a high affini ty for IAA. Its location and high affinity for IAA suggest that the cytoplasmic - nuclear ~inding protein is capable of detecting low intracellular concentration of auxin. Libbenga an~ Mennes (1987) suggest that the cytoplasmic-nuclear auxin-binding proteins are receptors functioning similar to the -steroid hormone receptors in animals.

10.4 BRASSINO STEROIDS AS CYTOKININ-BINDING PROTEINS

The most extensively characterized cytokinin-binding protein is CBF-l protein (cytokinin binding factor). CBF-l appears to be loosely associated with ribosomes since it is prepared by washing the ribosomal fraction with salt. This suggests that the CBF-l cytokinin complex might have a role in regulating the protein translation process. 8.8.4 Gibberellin and Abscisic acid-binding proteins There are no confirmed reports of high-affinity binding proteins for gibberellin, abscisicacid and ethylene. However, there is strong evidence for a high-affinity ABA-binding site on guard cell protoplasts. These sites are proteins located on the apoplastic surface of the plasma membrane.

10.5 BRASSINOSTEROIDS AS SECOND MESSENGERS IN PLANTS

The most promising second messengers in plants are calcium and the phosphoinositides.Calcium: Calcium controls many physiological processes in plants, including cell elongation arid division, protoplasmic streaming, the secretion and activity of various enzymes, hormone action and tactic and tropic responses. Plants also contain several calcium-binding proteins.

Among them calmodulin appears to be the dominant type.For calcium to function effectively as a second messenger, the cytosolic Ca2+concentration must be low and under metabolic control. Large amounts of calcium are stored in the Endoplasmic reticulum; the mitochondria and the large central vacuole. But the cytosolic Ca2+ concentration is kept low through the action of membrane-bound, calcium dependent ATPases. Activity of ATPase, the cytoplasmic Ca2+ concentration is under control of light and hormones (Fig. 10.5). In the cytosol,~a2+ reacts with and forms 'a complex, CaM.Ca2+. This serves to activate some enzymes. NAD 'kinases arid protein kinases are, stimulated by taM.Ca2+. NAD kinase catalyze the phosphorylation of NAD 'to 'NADP in the presence of.ATP~ Similarly, manybth~renzymes are activated by protein kinase-catalyzed phosphorylation.

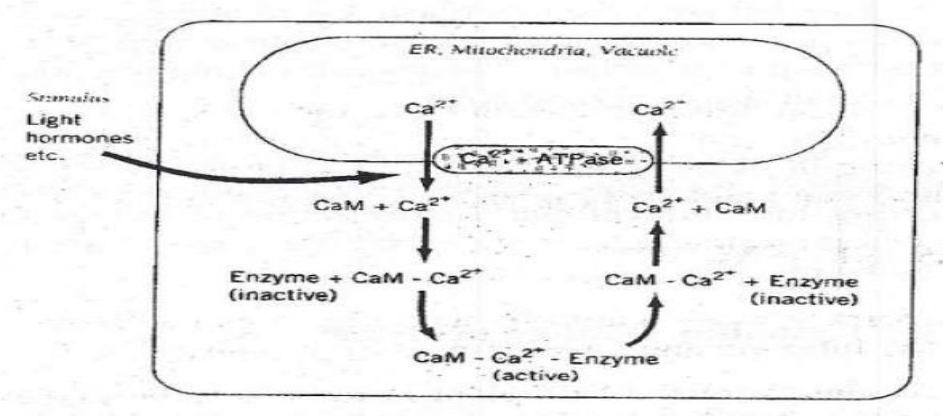
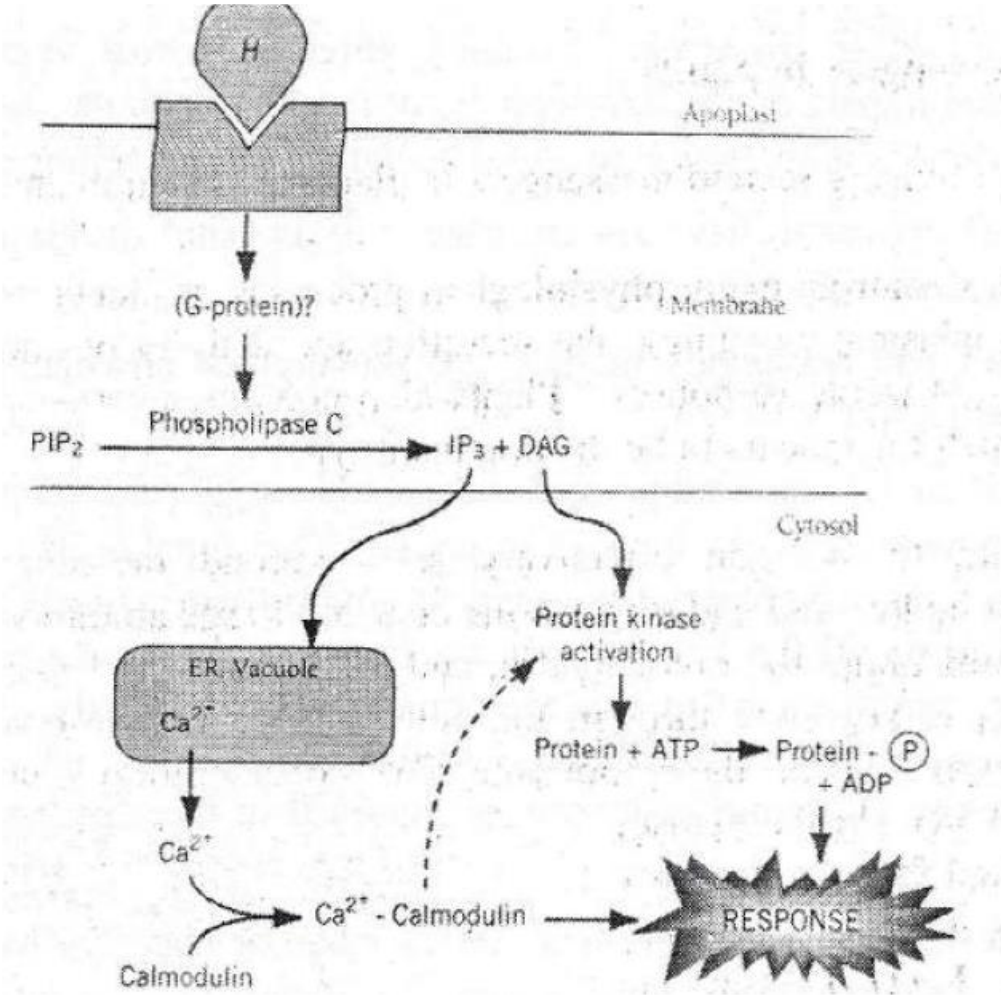


Fig. 10.5 Calcium as a second messager

Phosphdinositides:

Another second messenger in plants is the inositol triphosphate system. In ~his system, the hormone receptor-complex activatesa plasma membrane enzyme known as phospholipase C (Fig. 10.6). The hormone receptor complex may act through a G protein.

Phospholipase C catalyzes the break down of phosphotidylinositol bisphosphate (P,fP2), a membrane phospholipid, to inositol. triphosphate (IP3) and diacylglycerol (DAG). 'BothIP3 and pAG may function as second messengers. IP3 moves into the cytoplasm where it stimulates the release of calcium from the vacuole. Note that IP3 functions as a second messenger to mobilize ¥et another second messenger.icalcium. At the same time, DAG activates a particular protein kinase called protein kinaseC, Hydrolysis of PIP2 by phospholipase C, an increase in IP3 and bAG, transient increases in:cytosolic calcium and acti vation.of protein kinaseC. '



'Fig. 10,6 ,A model for thePhospholipase

In addition to calcium and the phosphoinositidcs, there are other second messengers in plants. They are acetylcholine and certain lipids. The 'study, of secondary messengers: .and ,hormone-initiated signal-transduction pathways in plant cells -is in its.infancy, but promises to be an important and exciting area of research in future. r . '• .:. '~~.

10.6 SUMMARY

Brassinosteroids appear to have an important role in plant development and may soon be considered a distinct class of hormones.Other possible plant hormones include the polyamines and the hypothetical hormones,florigen and vemalin. The events initiated by hormones can be (1) the initial signal perception, (2) a signal transduction pathway, and (3) the final response. The signal transduction pathway involves one or more second messengers that serve to amplify the original signal. Plant hormones appear to qualify on all three counts. It has been known for the past 100 years that plant hormones have significant effect on development. More recently, proteins that could servein the perception stage have been identified for auxin and cytokinin and ethylene. Lipidbased molecules and calcium appear to be involved in the signal transduction pathway in plants.

10.7 MODEL QUESTIONS.

1. Hormone binding proteins in plants
2. Polyamines
3. Give a detailed account on second messengers in plants.
4. Brassinosteroids

10.8 REFERENCE BOOKS

1. Introduction to Plant Physiology, 2nd edition, William G. Hopkins, John Wiley & Sons, Inc.
2. Plant Physiology. 3rd Ed., Salisbury and Ross, CBS Publishers and Distributors.
3. Advanced Plant Physiology, Malcolm B. Wilkins, English Language Book SocietylLongman.
4. Plant Physiology L. Taiz and E. Zeiger Sinauver Associates Inc., Sunderland, Massachusetts.

Prof A. Amrutha Valli

LESSON-11

CELL SIGNALLING, G PROTEINS AND SECOND MESSENGERS

OBJECTIVE:

To learn cell signaling, G proteins, and second messengers is to comprehend the fundamental mechanisms by which cells receive, process, and respond to information from their environment. This knowledge is crucial for understanding how biological processes—such as growth, immunity, and metabolism—are regulated at a molecular level

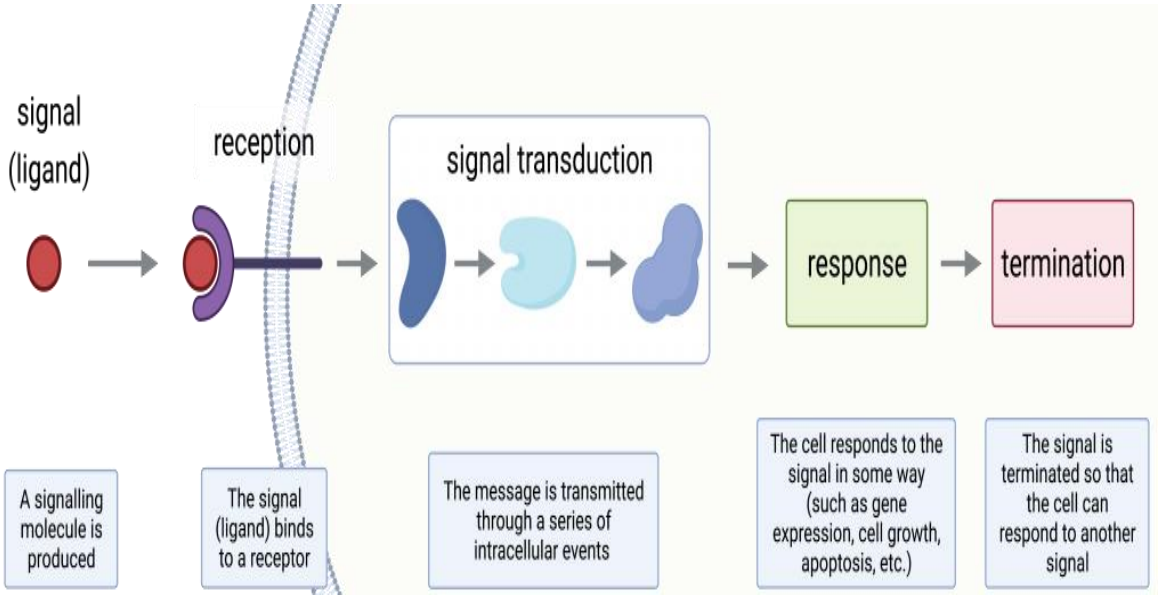
STRUCTURE OF THE LESSON:

11.1 INTRODUCTION

It is vital for individual cells to be able to interact with their environment. This is true for both a one-celled organism growing in a puddle and a large animal living on a savanna. In order to properly respond to external stimuli, cells have developed complex mechanisms of communication that can receive a message, transfer the information across the plasma membrane, and then produce changes within the cell in response to the message.In multicellular organisms, cells send and receive chemical messages constantly to coordinate the actions of distant organs, tissues, and cells. The ability to send messages quickly and efficiently enables cells to coordinate and fine-tune their functions.

11.2 STEPS OF CELL SIGNALLING

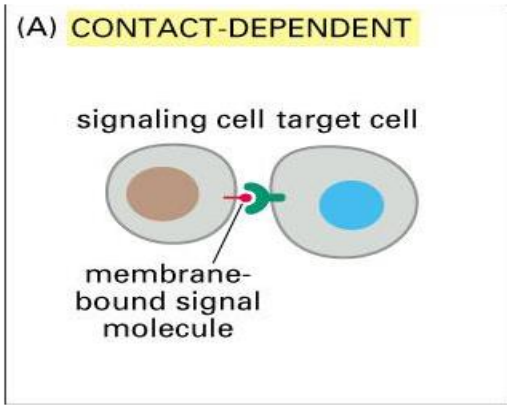
Cell signaling can be divided into five steps: signal, reception, signal transduction, response, and termination.



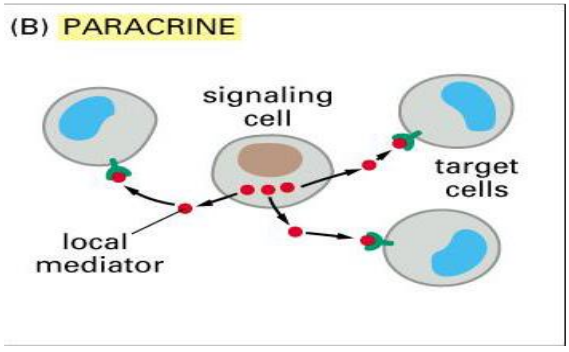
Cell signaling can be divided into five major steps: production of the signal, reception, signal transduction, response, and termination of the signal.

11.3 TYPES OF SIGNALS

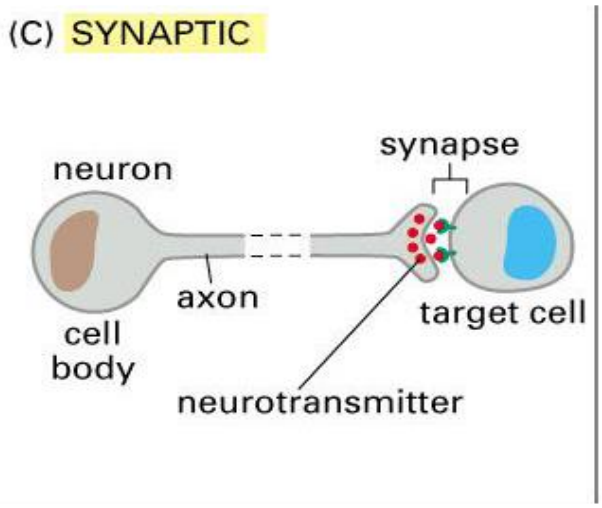
1. Contact dependent signaling requires cells to be in direct membrane-membrane contact. This is important during development and in immune responses. 6Four forms of intercellular signaling .



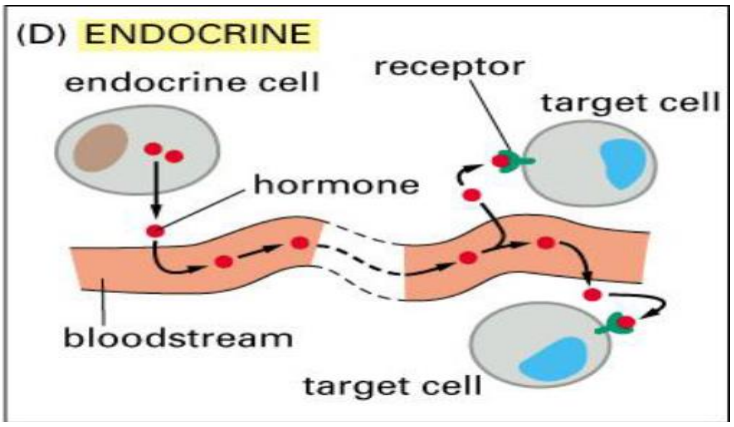
2. Paracrine signaling depends on local mediators that are released into the extracellular space and act on neighboring cells. E.g. nerve-muscle



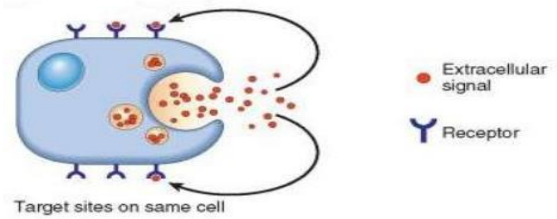
3. synaptic signaling is performed by neurons that transmit signals electrically along their axons and release neurotransmitters at synapses. Four forms of intercellular signaling



4. endocrine signaling depends on endocrine cells, which secrete hormones into the bloodstream for distribution throughout the body

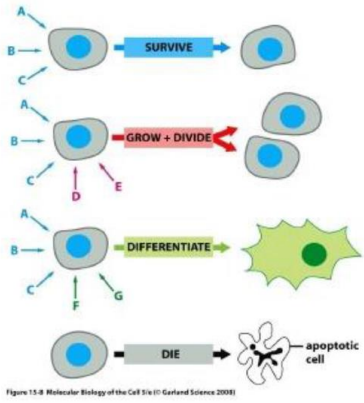


5. Autocrine signaling- cell that releases the signal is also the target



Autocrine Signalling

6. Each cell is programmed to respond to specific combinations of extracellular signals A cell may require multiple signals (A,B,C) to survive.



Each cell is programmed to respond to specific combinations of extracellular signals

7. Additional signals to grow and divide (D,E) or differentiate (F,G). If appropriate survival signals are deprived off, the cell undergoes apoptosis.
8. Extracellular signal molecules bind to specific receptors **:** Extracellular signal molecules include proteins, small peptides, amino acids, nucleotides, steroids, retinoids, fatty acid derivatives, NO, CO. •

Chemical signals are released by signalling cells in the form of small, usually volatile or soluble molecules called ligands. A ligand is a molecule that binds another specific molecule, in some cases, delivering a signal in the process. Ligands can thus be thought of as signaling molecules.Ligands interact with proteins in target cells, which are cells that are affected by chemical signals; these proteins are called receptors. Ligands and receptors exist in several varieties; however, a specific ligand will have a specific receptor that typically binds only that ligand.

11.4 SIGNALING LIGANDS

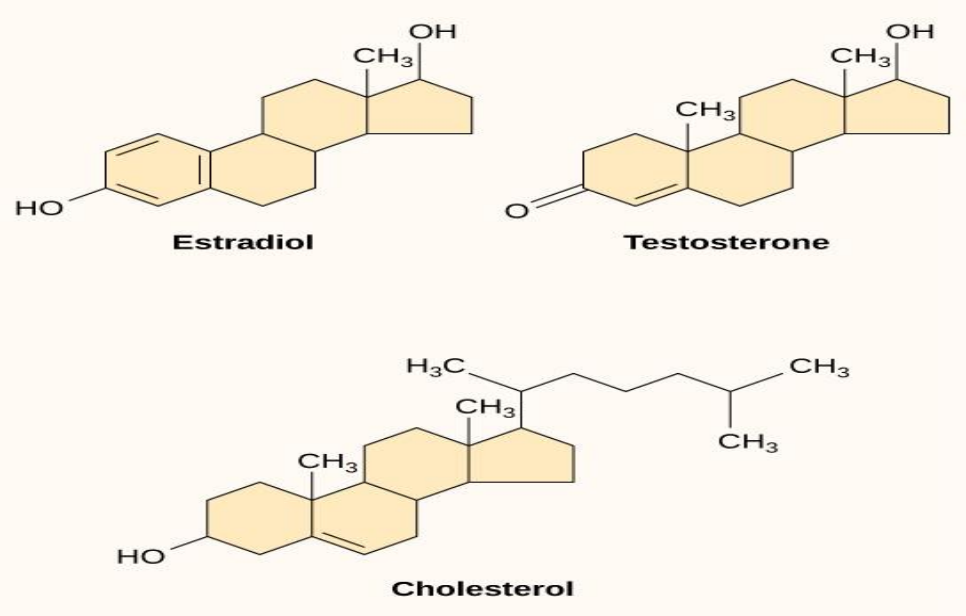
Produced by signaling cells and the subsequent binding to receptors in target cells, ligands act as chemical signals that travel to the target cells to coordinate responses. The types of molecules that serve as ligands are incredibly varied and range from small proteins to small ions like calcium (Ca^{2+}).

Small Hydrophobic Ligands

Small hydrophobic ligands can directly diffuse through the plasma membrane and interact with intracellular receptors. Important members of this class of ligands are the steroid hormones. Steroids are lipids that have a hydrocarbon skeleton with four fused rings; different steroids have different functional groups attached to the carbon skeleton. Steroids include the female sex hormone, estradiol, which is a type of estrogen; the male sex hormone, testosterone; and cholesterol, which is an important structural component of biological membranes and a precursor of steroid hormones. Other hydrophobic hormones include thyroid hormones and vitamin D. In order to be soluble in blood, hydrophobic ligands must bind to specific proteins while they are being transported through the bloodstream.

Water-Soluble Ligands

Hydrophilic ligands are polar and, therefore, cannot pass through the plasma membrane unaided. Sometimes they are too large to pass through the membrane at all. Instead, most water-soluble ligands bind to the extracellular domain of cell-surface receptors. This group of ligands is quite diverse and includes small molecules, peptides, and proteins.



Steroid hormones have similar chemical structures to their precursor, cholesterol. Because these molecules are small and hydrophobic, they can diffuse directly across the plasma membrane into the cell, where they interact with internal receptors.

11.5 RECEPTORS

Receptors are protein molecules inside the target cell or on its surface that bind ligand. We can divide receptors into two main classes, intracellular receptors and cell-surface receptors.

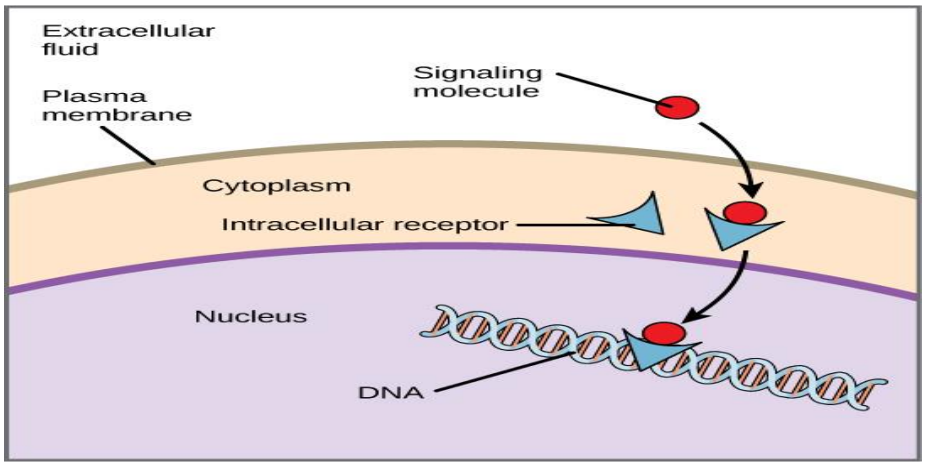
Intracellular receptors

Intracellular (or cytoplasmic) receptors, are found in the cytoplasm of the cell and respond to hydrophobic ligand molecules that are able to travel across the plasma membrane by simple diffusion. Once inside the cell, many of these molecules bind to proteins that act as regulators of mRNA synthesis (transcription) to mediate gene expression. Gene expression is the cellular process of transforming the information in a cell's DNA into a sequence of amino acids, which ultimately forms a protein.

When a ligand binds to an intracellular receptor, a conformational change is triggered that exposes a DNA-binding site on the protein. The ligand-receptor complex moves into the nucleus, then binds to specific regulatory regions of the chromosomal DNA and promotes the initiation of transcription. Intracellular receptors can directly influence gene expression without having to pass the signal on to other receptors or messengers.

Cell-Surface Receptors

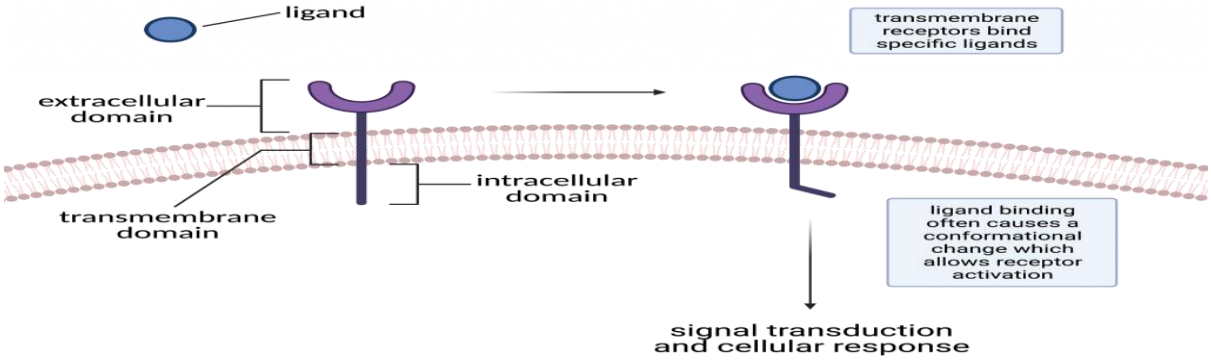
Cell-surface receptors, also known as transmembrane receptors, are cell surface, membrane-anchored (integral) proteins that bind to external ligand molecules. This type of receptor spans the plasma membrane and performs signal transduction, through which an extracellular signal activates an intracellular signal. Ligands that interact with cell-surface receptors do not have to enter the cell that they affect. Some cell-surface receptors are also called cell-specific proteins or markers because they are specific to individual cell types.



Hydrophobic signaling molecules typically diffuse across the plasma membrane and interact with intracellular receptors in the cytoplasm. Many intracellular receptors are transcription factors that interact with DNA in the nucleus and regulate gene expression.

Because cell-surface receptor proteins are fundamental to normal cell functioning, it should come as no surprise that a malfunction in any one of these proteins could have severe consequences. Errors in the protein structures of certain receptor molecules have been shown to play a role in hypertension (high blood pressure), asthma, heart disease, and cancer.

Each cell-surface receptor has three main components: an external ligand-binding domain called the extracellular domain, a hydrophobic membrane-spanning region called a transmembrane domain, and an intracellular domain inside the cell that can connect to a specific signal transduction pathway. The size and extent of each of these domains vary widely, depending on the type of receptor.

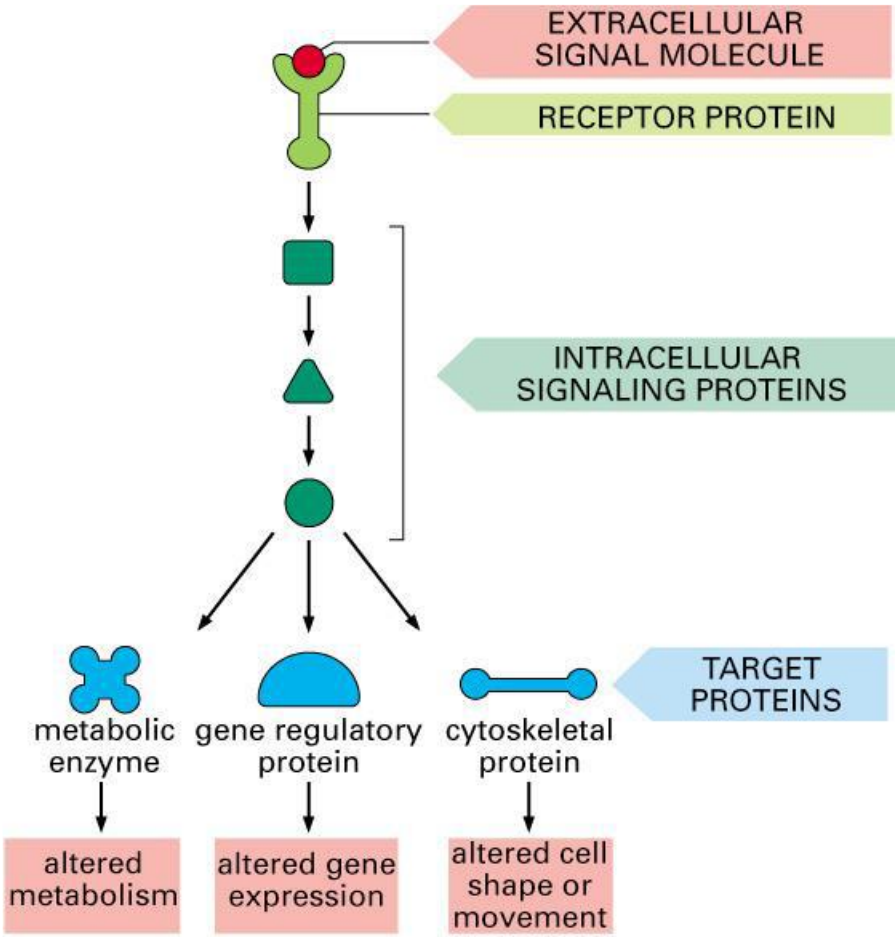


Transmembrane proteins can serve as receptors. When a ligand binds to the receptor, the receptor is activated and can elicit a specific cellular response, such as turning on or off transcription of certain genes.

Cell signaling is part of a complex system of communication that governs basic cellular activities and coordinates cell actions. The ability of cells to perceive and correctly respond to their microenvironment is the basis of development, tissue repair, and immunity as well as normal tissue homeostasis Errors in cellular information processing are responsible for diseases such as cancer, autoimmunity, and diabetes.

By understanding cell signaling, diseases may be treated effectively and, theoretically, artificial tissues may be created. These pathways depend on intracellular signaling proteins which process the signal and transmit the signal to appropriate intracellular targets. The targets at the end of signaling pathways are called effector proteins.

A simple intracellular signaling pathway activated by an extracellular signal molecule The signal molecule usually binds to a receptor protein in the PM of the target cell. The receptor activates one or more intracellular signaling pathways, involving a series of signaling proteins. Finally, one or more of the intracellular signaling proteins alter the activity of effector proteins and thereby the behaviourof the cell. Four forms of intercellular signaling

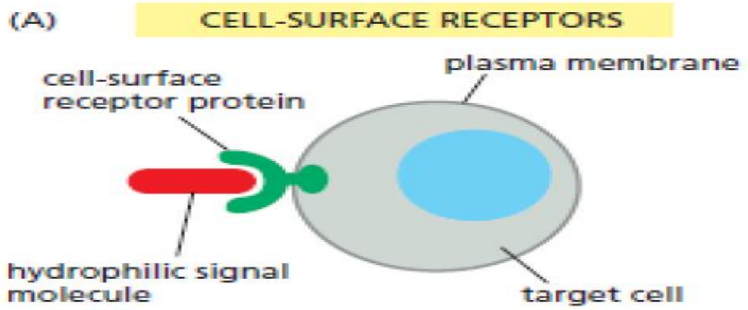


• Cells usually communicate with each other through extracellular messenger molecules.

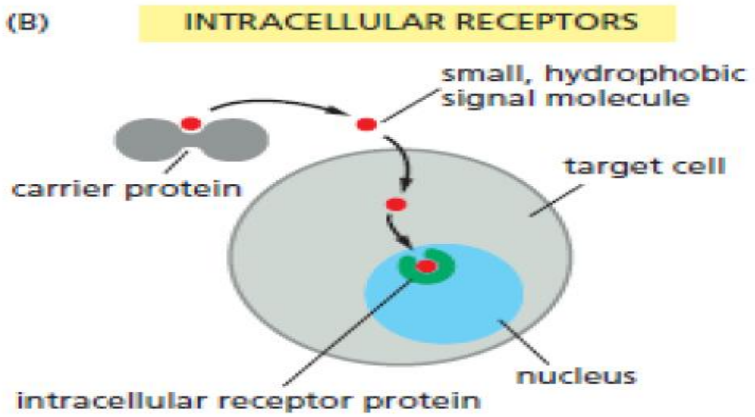
Extra cellular Receptor

Target cells respond by means of receptors. • Receptors are of two types:

1. Mostly, transmembrane proteins on the target-cell surface. When they bind to Extra cellular molecule (a ligand), and act as signal transducer, they become activated and generate various intracellular signals that alter the behaviour of cell.



2. Intracellular receptors-the signal molecule has to be small to diffuse across the PM and bind to receptor proteins inside the target cell-either in the cytosol or nucleus.



Cell surface receptors:

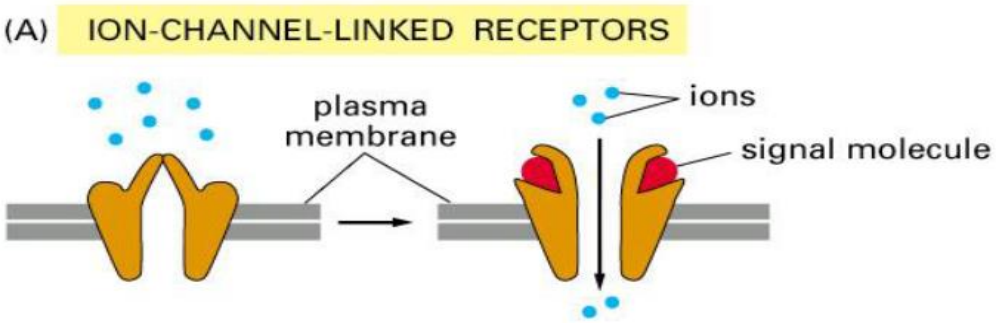
Cell-surface receptors include: ***ion-channel, G-protein, and enzyme-linked protein receptors***. The binding of these ligands to these receptors results in a series of cellular changes. These water soluble ligands are quite diverse and include small molecules, peptides, and proteins.

Cell surface receptors are three types

A. Ion -channel-linked receptors bind a ligand and open a channel through the membrane that allows specific ions to pass through.
B. G-protein-linked receptors bind a ligand and activate a membrane protein called a G-protein, which then interacts with either an ion channel or an enzyme in the membrane.
C. Enzyme-linked receptors are cell-surface receptors with intracellular domains an enzyme. that are associated with enzymes

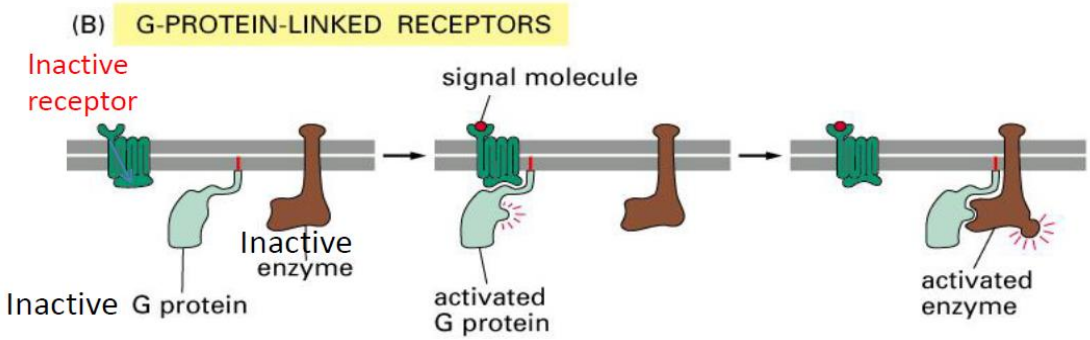
Ion channel-linked receptors

Also called transmittergated ion channels or ionotropic receptors. Found in nerve and muscle cells (electrically excitable). This type of mediated signaling is through neurotransmitters.



When a ligand binds to the extracellular region of the ionchannel-linked receptors, there is a conformational change in the receptor protein's structure that allows ions such as sodium, calcium, magnesium, and hydrogen to pass through

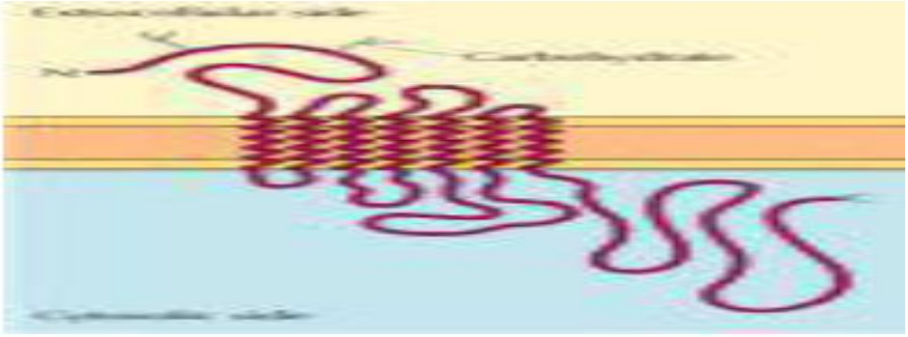
G-protein-linked receptors



Inactive receptor

The G protein-coupled receptors are characterized by seven membrane-spanning α helices .~45% of all pharmaceutical drugs are known to target GPCRs

G- prptein linked receptor is the tyrosinekinasereceptor. Signaling molecules bind to the extracellular domain of two nearby tyrosine kinase receptors, which then dimerize. The tyrosine kinase receptor transfersphosphategroups to tyrosine molecules on the intracellular domain of the receptors and can then transmit the signal to the next messenger within the cytoplasm

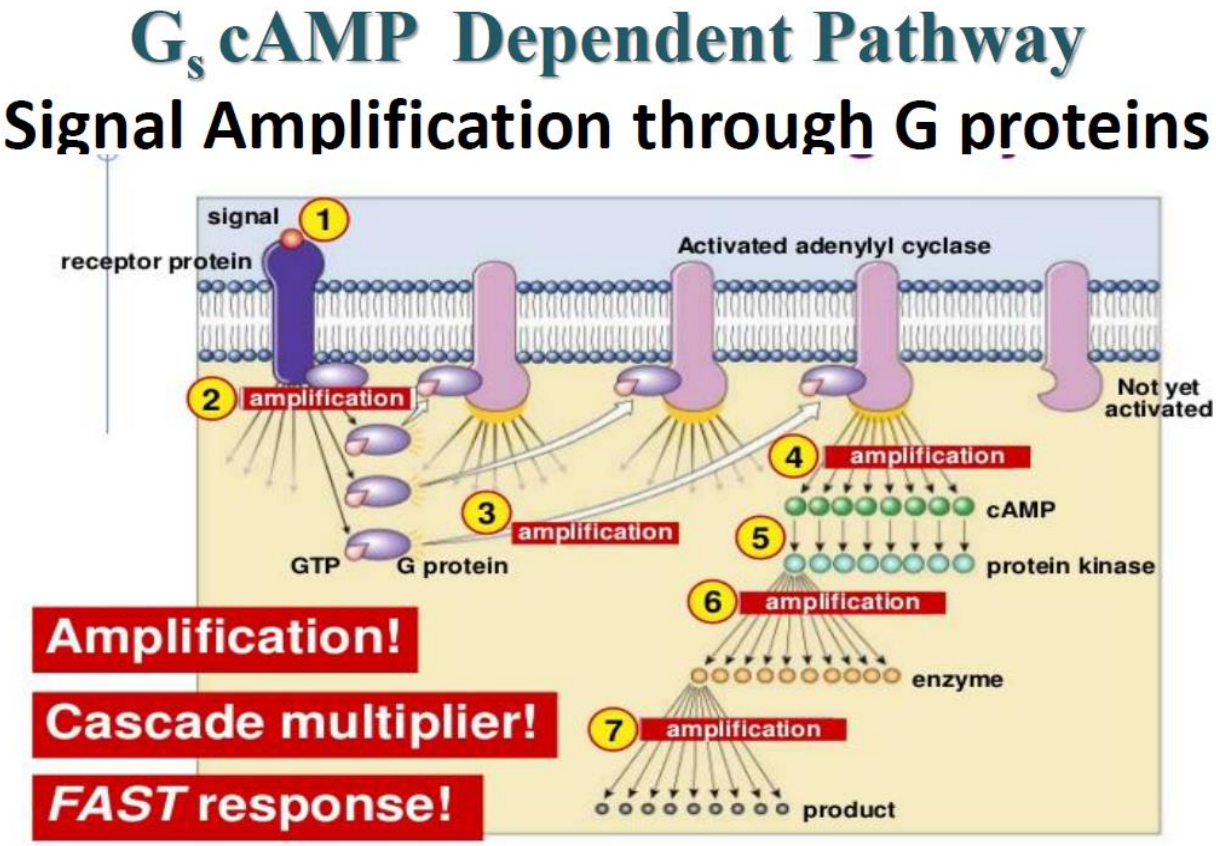


Signal molecules

- Biogenic amines: Adrenaline, noradrenaline, dopamine,, histamine, acetylcholine
- Amino acids and ions: Glutamate, Ca2+, GABA
- Lipids : prostaglandins, leukotrienes (produced in leukocytes by the oxidation of
- archidonic acid)
- Peptides / proteins : GnRH, angiotensin, bradykinin, thrombin, bombesin, glucagon, calcitonin, vasoactive intestinal peptides, PTH, FSH, LH, TSH
- Nucleotides : Adenosine nucleotides, adenine nucleotides, uridine nucleotides
- Others : Light, odorants, pheromones, opiates

G-protein-linked receptors

Robert Lefkowitz and Brian Kobilka: the 2012 Nobel Prize in Chemistry for groundbreaking discoveries that revealed the inner workings of G-protein–coupled receptors. The binding of ligands to the extracellular domain of these receptors induces a conformational change in the receptor and exposes a binding site for a G protein (bound to the inner face of the plasma membrane). G protein consists of α,β,γ subunits Heterotrimeric G proteins MammalianGprotein complexes are made up of 20 alpha (α) 6 beta (β) 12 gamma (γ) subunits. Betaand gammasubunits can form a stable dimeric complex referred to as the beta-gamma complex. In the resting state, α is bound to GDP.



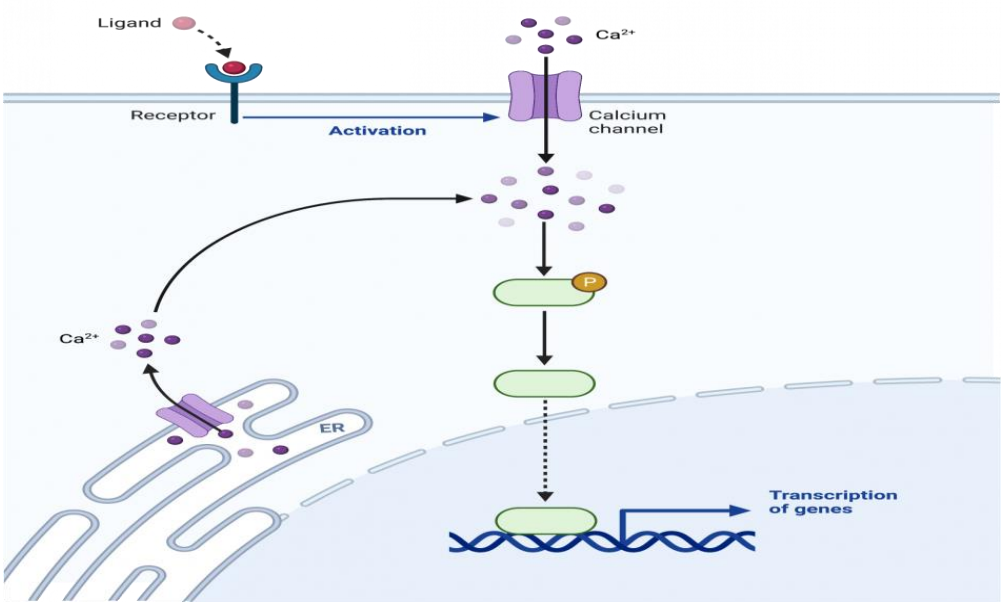
1. Ligand binding induces a conformational change in the receptor, such that the cytosolic domain of the receptor interacts with the G protein and stimulates the release of bound GDP from α subunit and its exchange for GTP.

2. The activated GTP-bound α subunit then dissociates from β and γ, which remain together and function as a βγ complex.
3. α subunit moves along the inner membrane and interact with another membrane bound protein 'primary effector' adenyl cyclase to elicit an intracellular response.
4. The activity of the α subunit is terminated by hydrolysis of the bound GTP, and the inactive α subunit (now with GDP bound) then reassociates with the βγ complex, ready for the cycle to start anew.• 'primary effector' creates a second messenger which may activate a 'secondary effector'protein kinase
5. The G protein associated with the epinephrine receptor is called Gs because its α subunit stimulates adenyl cyclase.

11.5 SECOND MESSENGERS

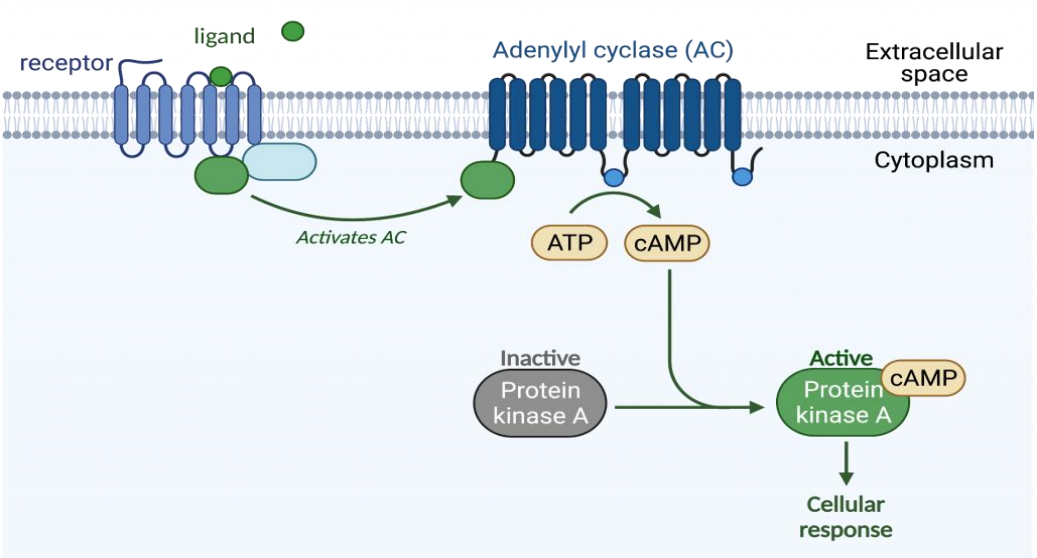
Second messengers are small molecules that propagate a signal after it has been initiated by the binding of a signaling molecule to its receptor. These molecules help to spread a signal through the cytoplasm by altering the behavior of certain cellular proteins.

Calcium ion is a widely used second messenger. The free concentration of calcium ions (Ca^{2+}) within a cell is very low because ion pumps in the plasma membrane continuously remove it. For signaling purposes, Ca^{2+} is stored in cytoplasmic vesicles, such as the endoplasmic reticulum, or accessed from outside the cell. When signaling occurs, ligand-gated calcium ion channels allow the higher levels of Ca^{2+} that are present outside the cell (or in intracellular storage compartments) to flow into the cytoplasm, which raises the concentration of cytoplasmic Ca^{2+}. The response to the increase in Ca^{2+} varies and depends on the cell type involved. For example, in the β-cells of the pancreas, Ca^{2+} signaling leads to the release of insulin, and in muscle cells, an increase in Ca^{2+} leads to muscle contractions. In other cells, it leads to transcription.

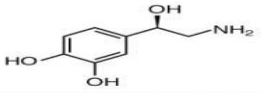
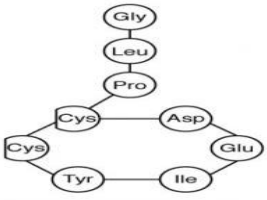
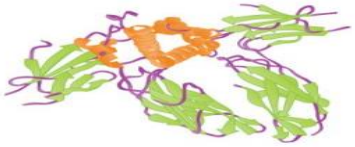
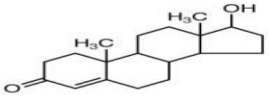
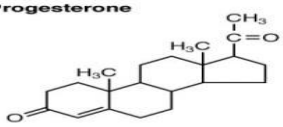


Calcium ions are a very common second messenger. When a ligand binds to a receptor, calcium is released from intracellular compartments and/or allowed to cross the membrane through calcium channels. (Calcium as a second messenger by Melissa Hardy is used under a Creative Commons Attribution-ShareAlike license. Created with BioRender.com).

Another second messenger utilized in many different cell types is cyclic AMP (cAMP). Cyclic AMP is synthesized by the enzyme adenylyl cyclase from ATP. The main role of cAMP in cells is to bind to and activate an enzyme called cAMP-dependent kinase (A-kinase). A-kinase regulates many vital metabolic pathways: It phosphorylates serine and threonine residues of its target proteins, activating them in the process. A-kinase is found in many different types of cells, and the target proteins in each kind of cell are different. Differences give rise to the variation of the responses to cAMP in different cells.



Protein kinase A is activated by the second messenger cyclic AMP (cAMP). When a ligand binds to the receptor, a subunit of a G-protein binds to adenylyl cyclase, which converts ATP to cAMP. cAMP can then bind to Protein Kinase A, activating it.

Hormone Class	Components	Example(s)
Amine Hormone	Amino acids with modified groups (e.g. norepinephrine's carboxyl group is replaced with a benzene ring)	**Norepinephrine** 
Peptide Hormone	Short chains of linked amino acids	**Oxytocin** 
Protein Hormone	Long chains of linked amino acids	**Human Growth Hormone** 
Steroid Hormones	Derived from the lipid cholesterol	**Testosterone**  **Progesterone** 

Hormones can be derived from amino acids or lipids.
(Figure by OpenStax is used under a Creative Commons Attribution license).

11.6 SUMMARY

Signal transduction involves a cell responding to a ligand, a signaling molecule, by binding it to a specific receptor, which triggers a cascade of intracellular events to change cell behavior. Ligands can be water-soluble (binding to cell-surface receptors) or lipid-soluble (binding to intracellular receptors). Once the ligand binds, the receptor changes shape, initiating a signal that can lead to altered gene expression, enzyme activation, or changes in cell metabolism, proliferation, or movement. S

11.7 Model questions

1. Write about different types of Signals
2. Give a brief account on Receptors
3. Explain different types of Signaling ligands

11.8 Suggested readings

1. Introductory plant physiology - G.R. Noggle and G.J.Fritz. Prentice Hall of India – New Delhi.
2. Plant Physiology - R.M. Devlin and F.H.Witham - CBS Publishers and distributors - New Delhi
3. P!ant Physiology - F.B. Salisbury and C.W.Ross - CBS Publishers - New Delhi
4. plant Physiology -l.Taiz and E.Zeiger - Sinauer Associates, Inc., Publishers, Suderland, Massachusetts.
5. Introduction to plant Physiology - W.G. Hopkins. John Wiley & Sons. Inc - New York.

Prof A. Amrutha Valli

LESSON -12

TWO COMPONENT SENSOR REGULATOR SYSTEM IN BACTERIA AND PLANTS

OBJECTIVES :

To learn about two-component sensor regulator systems is to understand how bacteria and plants detect and respond to changes in their environment. These systems are crucial for processes such as **survival, growth, and development**.

STRUCTURE OF THE LESSON:

12.1 INTRODUCTIONS

In molecular biology, two-component systems serve as a basic stimulus-response coupling mechanism allowing organisms to sense and respond to changes in many different environmental conditions. They typically consist of a membrane -bound histidine kinase that senses a specific environmental stimulus and a corresponding response regulator that mediates the cellular response. Two component signaling systems are widely occurring in prokaryotes whereas only a few two-component systems have been identified in eukaryotic organisms.

The two-component regulatory system includes a membrane component and a cytoplasmic component. Outside the cell, the sensor domain of the kinase detects an environmental change, which leads to phosphorylation of the transmitter domain.

12.2 TWO-COMPONENT SYSTEM IN BACTERIA

A two-component system in bacteria is a signal transduction pathway that allows bacteria to sense and respond to environmental changes through a pair of proteins: a sensor kinase and a response regulator. The sensor kinase detects an external signal, autophosphorylates itself,

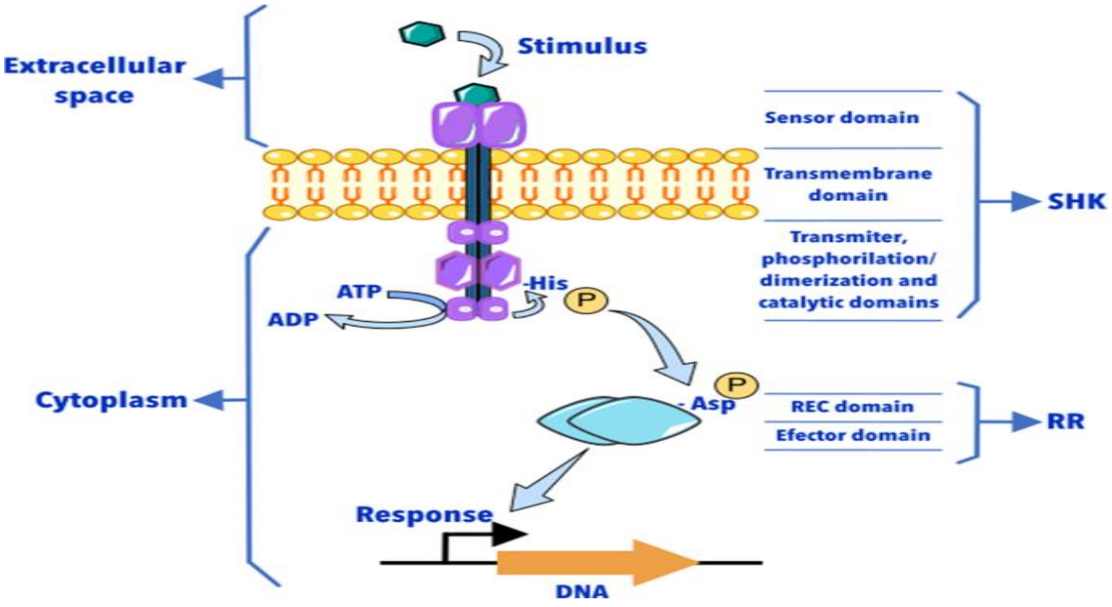
and then transfers a phosphate group to the response regulator. This phosphorylation activates the response regulator, which in turn modifies gene expression to help the bacterium adapt to the new condition, such as changes in osmolarity, nutrients, or the presence of antibiotics

12.2.1Components and process ;

Sensor Kinase:
This is the "sensor" protein, often a membrane-bound histidine kinase, that detects an environmental stimulus. Upon sensing the signal, it becomes autophosphorylated, meaning it adds a phosphate group to itself.

Response Regulator:
After being phosphorylated by the sensor kinase, the response regulator is activated.

3.Signal Transduction:
The activated response regulator then functions as a transcription factor, binding to specific genes and either activating or repressing their transcription. This changes the bacterial cell's protein expression to match the new environmental demands.



12.2.2 Examples of functions

Osmoregulation: The EnvZ/OmpR system in E. coli regulates the expression of outer membrane porins in response to changes in the external salt concentration.

Nutrient Transport: The KdpD/KdpFABC system regulates the expression of genes involved in potassium transport.

Chemotaxis: Some two-component systems are involved in chemotaxis, where they help the bacterium move toward or away from a chemical stimulus by controlling flagellar rotation.
Antibiotic Resistance: Many two-component systems are triggered by antibiotics and can activate defenses, modify cell permeability, or increase biofilm formation to promote resistance.

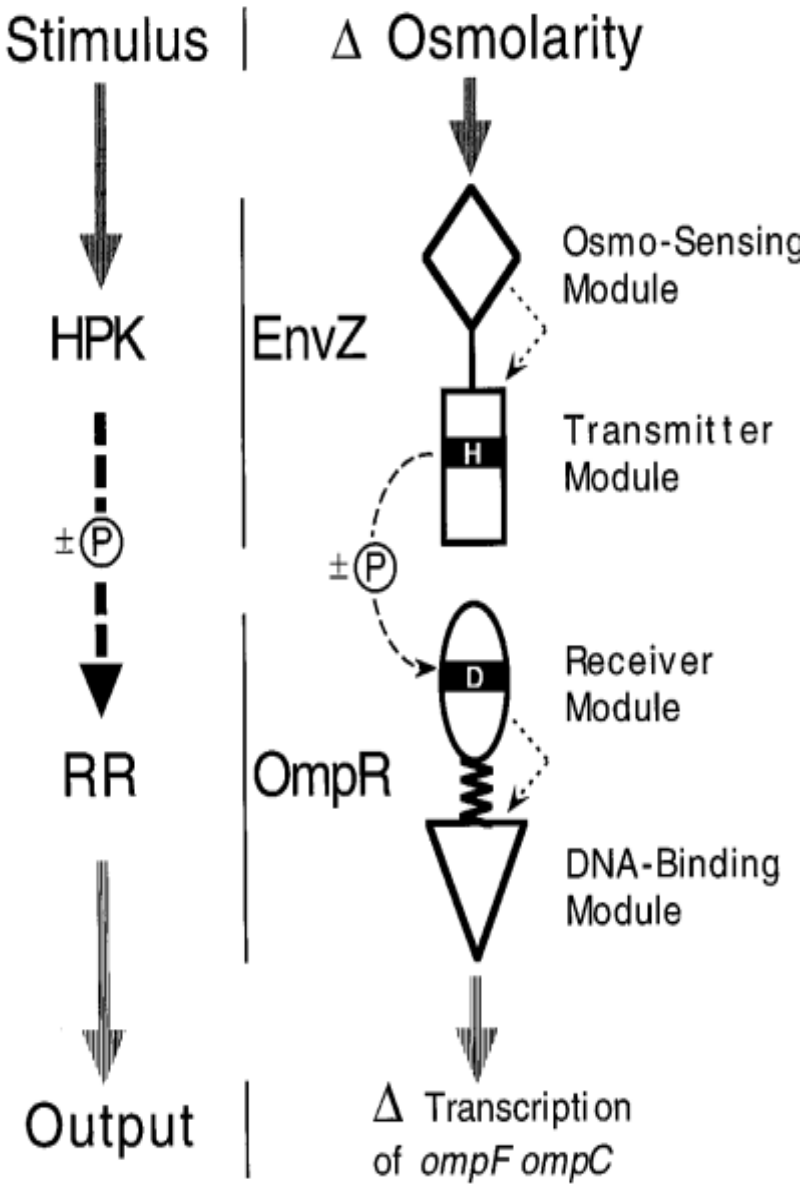


Figure . Example of a basic two-component system. The E. coli osmolarity-response system consists of an HPK osmosensor (EnvZ) and an RR transcription factor (OmpR) (Pratt and Silhavy, 1995). EnvZ autophosphorylates using ATP as the phosphate donor. The phosphate from the transmitter module of EnvZ is then transferred to an Asp residue in the receiver module of OmpR, thereby affecting the promoter interactions of the OmpR DNA-binding module, which regulates the transcription of two porin genes, ompF and ompC. Changes in osmolarity are perceived by the amino-terminal module of EnvZ. In response to such changes, EnvZ changes the level of phosphorylated OmpR. The dotted lines depict intra-protein regulatory interactions. The dashed line depicts phosphorylation/ dephosphorylation events. P, Phosphoryl group; H, His; D, Asp.

The basic two-component system involves a sensor kinase, or HPK, as well as an RR. As depicted in Figure , the role of the HPK is to direct phosphorylation of its cognate RR in

response to a specific environmental signal; this phosphorylation regulates the activity of the RR. Some bacteria make extensive use of such systems. For example, inspection of the complete genome of E. coli indicates that over 30 distinct HPK-RR circuits operate in this single bacterium. Basic Local Alignment Search Tool (BLAST) searches of the Mycoplasma genetilium genome database, however, revealed no likely HPK homologs, suggesting that not all prokaryotes utilize two-component systems as extensively as E. coli. Similar surveys of other sequence databases indicate that while some eukaryotes (e.g. Arabidopsis thaliana) may have a number of two-component systems, others (e.g. Saccharomyces cerevisiae) appear to have only a single two-component system. Here we outline some of the basic characteristics common to the large families of two-component HPKs and RRs.

Sensor HPKs

In several respects, HPKs are similar to the well-defined family of receptor Tyr kinases .HPKs operate as dimers and autophosphorylate; they are associated with the cytoplasmic membrane, usually via one or two membrane-spanning sequences; and they typically contain extracellular sensory input modules fused to the protein kinase catalytic module (Bourret et al., 1991). This arrangement makes it easy to envision environmental stimuli impinging on the HPK in a manner that regulates its kinase activity. However, there are relatively few cases in which we have much understanding of the actual ligands that interact directly with the HPKs, and in several cases a distinct protein serves as the primary receptor for the stimulus . Because of this, it has been difficult to determine the exact relationship between signal perception and catalytic activity of the HPK (e.g. whether the signal stimulates HPK activity). Despite the general mechanistic similarities shared by HPKs and other types of protein kinases, sequence analysis indicates that HPKs are only distantly related to Tyr kinases and Ser/Thr kinases. There are also operational features that distinguish HPKs from other protein kinases. First, HPKs do not catalyze direct transfer of a phosphate from ATP to their "substrate" RR; rather, each HPK must first autophosphorylate, and then the phosphoryl group from HPK-P is passed to the RR. A second difference is that the site of HPK autophosphorylation is a His residue, and the site of RR phosphorylation is an Asp residue . The energetics and chemical stabilities of phospho-His and phospho-Asp differ significantly from those of "more traditional" phospho-amino acids (phospho-Tyr, phosphoSer, and phospho-Thr) . Several hundred HPKs (some well characterized, some surmised based on sequence analysis) have been found in bacteria, and amino acid sequence comparisons have identified a common 250-amino acid "transmitter module" in each of these. This module is thought to encompass the autokinase active site and, in most cases, the Hisphosphorylation site. Excluding sequences of closely related homologs, the transmitter modules from any two HPKs typically share 20 to 50% sequence identity (average sequence identity, 25%). Five blocks of 5 to 10 amino acids with higher conservation have been identified in most transmitter modules. Some HPKs also have phosphatase activities, i.e. they can catalyze dephosphorylation of their cognate RRs This dephosphorylation appears to involve a mechanism that is distinct from simple reversal of the HPK-RR phosphotransfer reaction (Hsing and Silhavy, 1997).

RRs

The sensor HPK regulates the activity of a cytoplasmic RR by directing its phosphorylation as depicted in Figure 1. GenBank now contains over 400 different examples of RRs. Analysis of the amino acid sequences of known and suspected RRs has established two general themes: (a) RRs have an approximately 110-amino acid domain referred to as a "receiver module" that contains the Aspphosphorylation site; and (b) most RRs are two-domain proteins in which the receiver module is fused to a second domain having some kind of

output or effector activity (Parkinson and Kofoid, 1992). In many cases, the output domain is a DNA-binding module whereby the RR functions as a transcription factor, and Asp phosphorylation serves to control its ability to either bind its target DNA sequence or interact with other components of the transcription machinery (Hakenbeck and Stock, 1996). There are also RRs that have nothing to do with transcription. For example, E. coli CheB demethylates the chemotaxis-receptor proteins, and phosphorylation of the

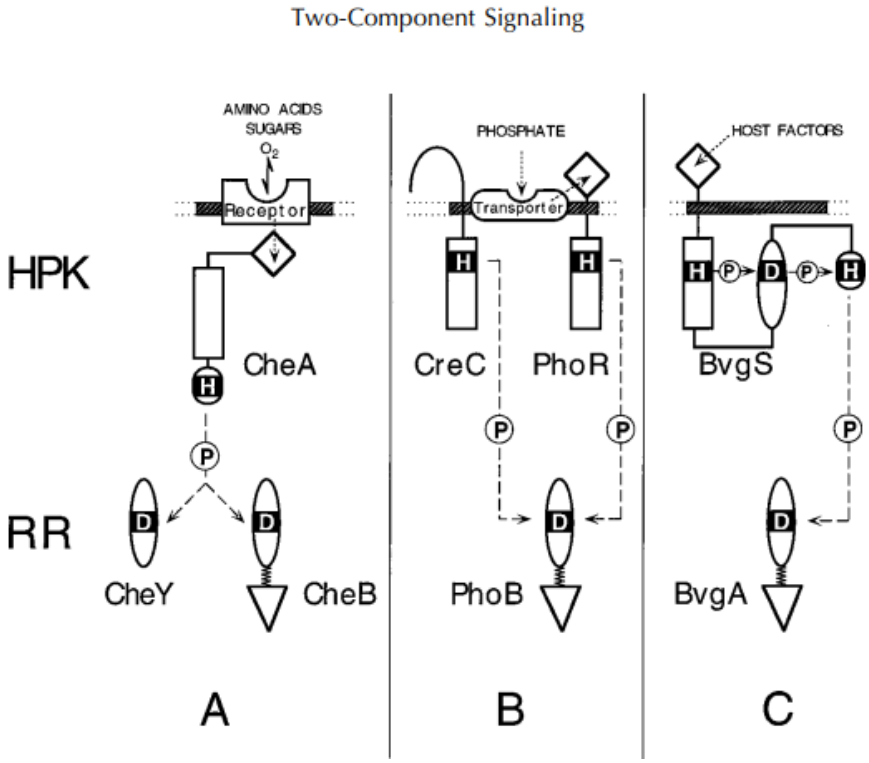


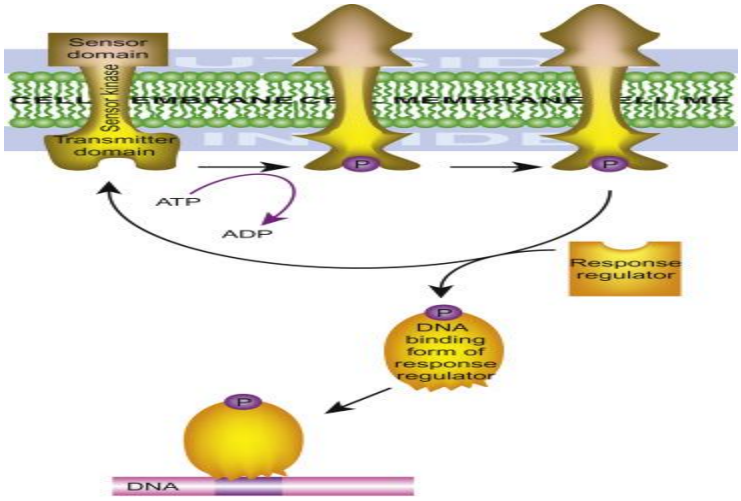
Figure . Examples of diversity in the two-component system. In A and B, a cell-surface receptor or transport protein is responsible for direct binding/detection of a stimulus, and this information is then conveyed to the appropriate HPK via protein-protein interactions. A, Part of the E. coli chemotaxis signaling pathway, a system in which the CheA HPK phosphorylates either of two different RRs; the CheY RR promotes changes in swimming direction, whereas the CheB RR promotes sensory adaptation (Bourret et al., 1991; Stock et al., 1991). The location of the phosphorylation site in CheA is atypical in that it is located outside of the transmitter module (Parkinson and Kofoid, 1992). B, Basic elements of the E. coli Pho(phosphate) system, which is responsible for controlling gene expression in response to phosphate availability. In this system, two distinct HPKs, PhoR and CreC, can phosphorylate the RR transcription factor PhoB. CreC responds to the intracellular concentration of some unknown metabolite, whereas PhoR is regulated by a phosphate-specific transport system (Wanner, 1994). C, BvgS, a hybrid HPK that regulates expression of virulence determinants of the human pathogen Bordetella pertussis in response to as-yet-unknown host factors. BvgS undergoes His to Asp to His phosphotransfer prior to phosphorylation of the RR transcription factor BvgA (Uhl and Miller, 1996). H, His; P, phosphoryl group; D, Asp.

CheB receiver module serves to enhance this activity (Fig. 2A) (Lupas and Stock, 1989). In the case of E. coli SprE, the output module regulates the activity of a protease (Pratt and Silhavy, 1996). Thus, the basic conformational changes associated with receiver

phosphorylation are able to control a variety of activities (Lowry et al., 1994). If one excludes sequences of closely related homologs (e.g. NRI from two closely related bacterial species), receiver modules from any two RRs share sequence identity at only 20 to 30% of the positions, but all receiver modules are thought to have a similar three-dimensional structure . X-ray crystal structures and/or NMRderived three-dimensional structures have been obtained for CheY , Spo0F , and NarL proteins. These structures indicate a common ab protein structure for the receiver modules in RRs, with the phosphorylation site located in the loop connecting two of the central strands of b sheet that comprise the core of the receiver module structure. The three-dimensional structures of receiver modules are strikingly similar to that of the small GTP-binding protein Ras. This similarity is especially interesting in view of the ability of Ras to control MAPK pathways in several eukaryotic systems .

12.3 PLANT TWO-COMPONENT SYSTEMS (TCS)

Plant two-component systems (TCS) are a signal transduction network that enables plants to sense and respond to environmental stimuli. Unlike the classic bacterial system of two components, plant TCS typically involves three components—hybrid histidine kinases, histidine phosphotransfer proteins, and response regulators—linked by a His-to-Asp phosphorelay. These systems are crucial for processes like sensing hormones like cytokinin, responding to ethylene, regulating growth, and responding to osmotic stress.



12.3.1 Components of the plant TCS

Hybrid Histidine Kinases (HKs):
These are sensor proteins that receive a signal, autophosphorylate, and then transfer a phosphate group to the next component.

Histidine Phosphotransfer Proteins (HPTs):
These act as intermediates, relaying the phosphate group from the HK to the response regulator in a series of steps.

Response Regulators (RRs):
These are the final components that receive the phosphate group, which then triggers a cellular response, often by altering gene expression.

12.3.2 Key functions

Hormone signaling: TCS plays a critical role in sensing and transducing signals from hormones like cytokinin and ethylene.

Osmosensing: They help plants respond to changes in water and solute concentration.

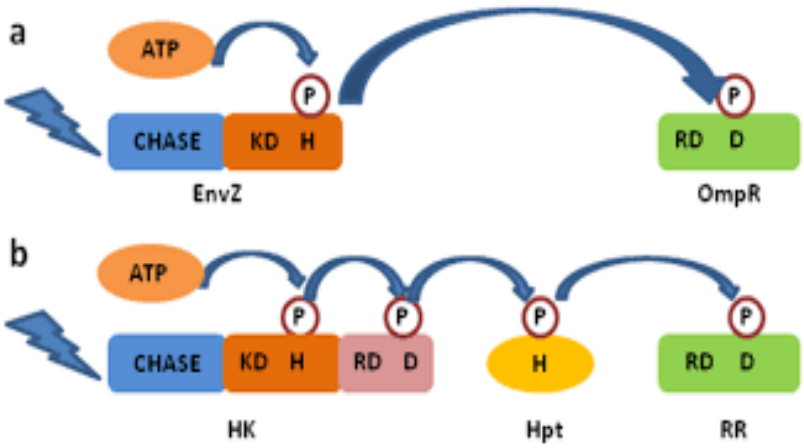
Development: They are involved in various developmental processes, such as megagametogenesis in Arabidopsis and flowering in rice.

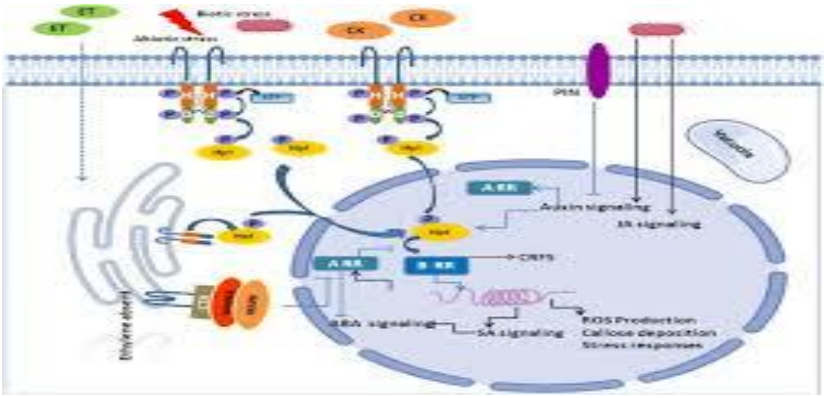
Environmental responses: TCS helps integrate signals from different phytohormones to regulate stress responses, like stomatal closure during drought.
Circadian clock: Two-component-like elements are components of the plant's circadian clock system.

12.4 SIGNALING PATHWAY

1. A signal (like a hormone) binds to the input domain of the histidine kinase (HK).
2. The HK autophosphorylates by transferring a phosphate from ATP to a histidine residue.
3. The phosphate is then transferred from the HK to the histidine phosphotransfer protein (HPT).
4. The HPT transfers the phosphate group to a response regulator (RR).
5. The phosphorylation of the RR triggers a downstream response, such as a change in gene transcription.

In plants, the "two-component system" (TCS) is an essential signal transduction pathway that enables responses to various stimuli, particularly **plant hormones** (cytokinins and ethylene), **environmental stresses** (drought, salinity, cold), and **light**. Unlike the simpler two-protein systems in bacteria, plants typically use a more complex **multistep phosphorelay** system involving three main components:





- **Hybrid Histidine Kinases (HKs):** These proteins, often located in the cell membrane or endoplasmic reticulum, act as the primary sensors for external or internal signals. Upon perceiving a signal (e.g., cytokinin binding), the HK undergoes autophosphorylation on a conserved histidine residue. It then transfers the phosphate group intramolecularly to a receiver domain within the same protein.

- **Histidine Phosphotransfer Proteins (HPts):** These are small, mobile proteins located primarily in the cytoplasm that act as an intermediate shuttle for the phosphate group. The phosphate is transferred from the HK's receiver domain to a conserved histidine residue on the HPt protein. HPts can move into the nucleus to continue the signal cascade.

- **Response Regulators (RRs):** These proteins receive the phosphate group from the HPts at a conserved aspartate residue in their receiver domain. RRs are typically categorized into types A, B, and C based on their structure and function.

Type-B RRs act as transcription factors that move into the nucleus and bind to specific DNA sequences to activate the expression of target genes.

Type-A RRs generally act as negative regulators of the pathway, often creating a negative feedback loop to limit the signal.

This multistep process (His → Asp → His → Asp) allows for signal amplification, integration, and fine-tuning, providing a sophisticated mechanism for plants to adapt their growth, development, and stress responses to dynamic environmental conditions.

Signal transduction occurs through the transfer of phosphoryl groups from adenosine triphosphate (ATP) to a specific histidine residue in the histidine kinases (HK). This is an autophosphorylation reaction. Subsequently the histidine kinase catalyzes the transfer of the phosphate group on the phosphorylated histidine residues to an aspartic acid residue on the response regulator (RR). Phosphorylation causes the response regulator's conformation to change, usually activating an attached output domain, which then leads to the stimulation (or repression) of expression of target genes. The level of phosphorylation of the response regulator controls its activity. Some HK are bifunctional, catalysing both the phosphorylation and dephosphorylation of their cognate RR. The input stimuli can regulate either the kinase or phosphatase activity of the bifunctional HK.

Two-component signal transduction systems enable bacteria to sense, respond and adapt to a wide range of environments, stressors and growth conditions. Some bacteria can contain as many as 200 two-component systems that need tight regulation to prevent unwanted cross-talk. These pathways have been adapted to respond to a wide variety of stimuli, including nutrients, cellular redox state, changes in osmolarity, quorum signals, antibiotics, temperature, chemo attractants, pH and more. In E. coli the EnvZ/OmpR osmoregulation system controls the differential expression of the outer membrane porin proteins OmpF and OmpC. The KdpD sensor kinase proteins regulate the kdpFABC operon responsible for potassium transport in bacteria including E. coli and Clostridium acetobutylicum. The N-terminal domain of this protein forms part of the cytoplasmic region of the protein, which may be the sensor domain responsible for sensing turgor pressure.

System Variants

A variant of the two-component system is the phospho-relay system. Here a hybrid HK autophosphorylates and then transfers the phosphoryl group to an internal receiver domain, rather than to a separate RR protein. The phosphoryl group is then shuttled to histidine phosphotransferase (HPT) and subsequently to a terminal RR, which can evoke the desired response.

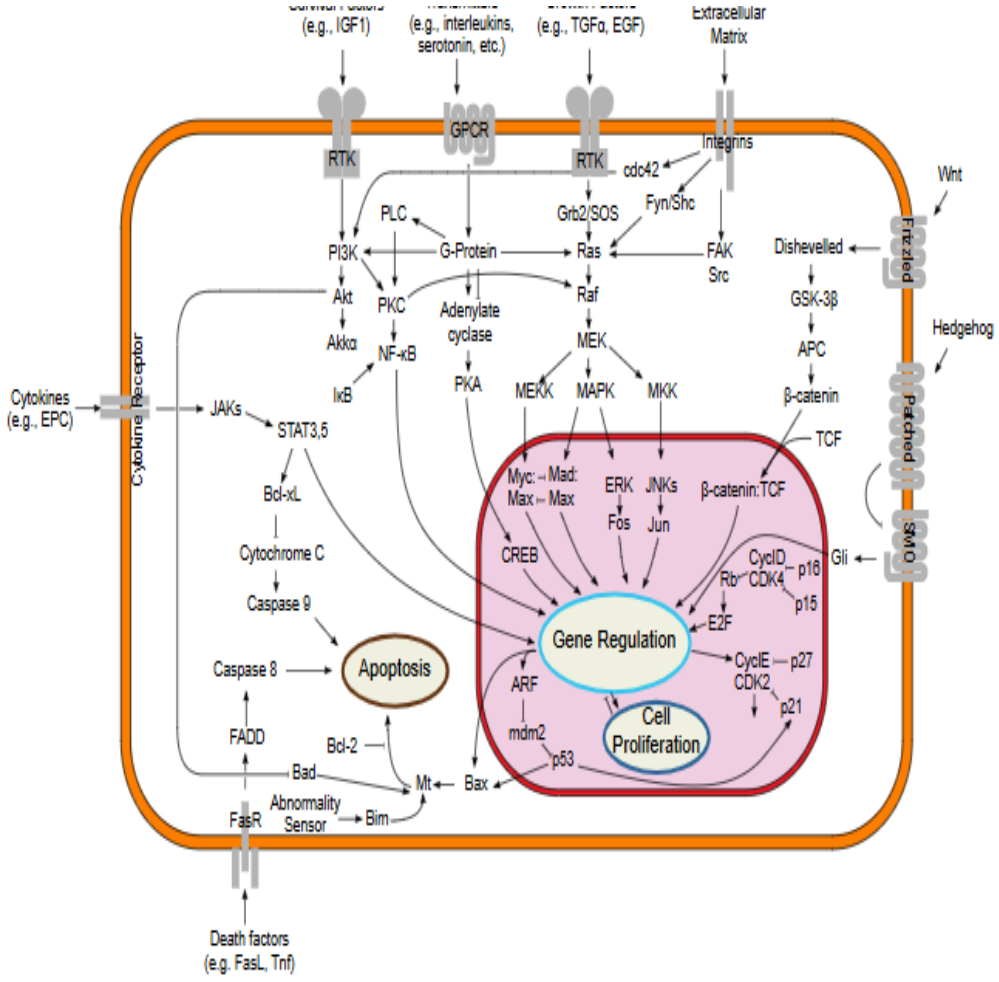


Figure: **Signal transduction**: An overview of major signal transduction pathways.

Signal transducing histidine kinases are the key elements in two-component signal transduction systems. Examples of histidine kinases are EnvZ, which plays a central role in osmoregulation, and CheA, which plays a central role in the chemotaxis system. Histidine kinases usually have an N-terminal ligand-binding domain and a C-terminal kinase domain, but other domains may also be present. The kinase domain is responsible for the autophosphorylation of the histidine with ATP, the phosphotransfer from the kinase to an aspartate of the response regulator, and (with bifunctional enzymes) the phosphotransfer from aspartyl phosphate back to ADP or to water. The kinase core has a unique fold, distinct from that of the Ser/Thr/Tyr kinase superfamily.

HKs can be roughly divided into two classes: orthodox and hybrid kinases. Most orthodox HKs, typified by the Escherichia coli EnvZ protein, function as periplasmic membrane receptors and have a signal peptide and transmembrane segment(s) that separate the protein into a periplasmic N-terminal sensing domain and a highly conserved cytoplasmic C-terminal kinase core. Members of this family, however, have an integral membrane sensor domain. Not all orthodox kinases are membrane bound, e.g., the nitrogen regulatory kinase NtrB (GlnL) is a soluble cytoplasmic HK. Hybrid kinases contain multiple phosphodonor and phosphoacceptor sites and use multi-step phospho-relay schemes instead of promoting a single phosphoryl transfer. In addition to the sensor domain and kinase core, they contain a CheY-like receiver domain and a His-containing phosphotransfer (HPt) domain.

The Hpr Serine/threonine kinase PtsK is the sensor in a multicomponent phosphorelay system in control of carbon catabolic repression in bacteria. This kinase is unusual in that it recognizes the tertiary structure of its target and is a member of a novel family unrelated to any previously described protein phosphorylating enzymes. X-ray analysis of the full-length crystalline enzyme from Staphylococcus xylosus at a resolution of 1.95 A shows the enzyme to consist of two clearly separated domains that are assembled in a hexameric structure resembling a three-bladed propeller. The blades are formed by two N-terminal domains each, and the compact central hub assembles the C-terminal kinase domains.

12.5. SUMMARY

Two-component signal transduction systems enable bacteria to sense, respond, and adapt to a wide range of environments, stressors, and growth conditions.Signal transduction can occur through the transfer of phosphoryl groups from adenosine triphosphate (ATP) to a specific histidine residue in the histidine kinases (HK).A variant of the two-component system is the phospho-relay system. The basic two-component system involves two large families of signaling modules that build upon a His-to-Asp phosphotransfer theme. Bacteria display numerous variations on this theme, illustrating the flexibility of the system. There is growing evidence, including a number of unpublished reports, that two-component regulators and distant relatives play important sensory-response roles in eukaryotes. These eukaryotic systems reveal further diversification of the two-component-based circuitry, most notably in the regulation of MAPK modules. Although quite a lot has been learned about how two-component systems operate, there remain numerous fundamental questions in both eukaryotic and prokaryotic systems; for example: How is HPK activity regulated by sensory input? What is the nature of the structural change resulting from receiver module phosphorylation, and how does this change result in the activation/deactivation of output activity? Are there “one-component” systems in which an “orphan” receiver module or transmitter module operates without a partner? In cells containing multiple two-component systems that respond to different stimuli, how is specificity maintained so as to minimize inappropriate “cross-

talk"? Are there examples of two-component systems in which the transmitter and receiver modules direct protein-protein interactions but do not involve protein phosphorylation? As more and more two-component systems are discovered, and as the number of researchers in this field grows, we look forward to the resolution of these issues, as well as to further surprises from these versatile signaling modules.

12.5 MODEL QUESTIONS

1. Two-component system in bacteria
2. Components and process ;
3. Examples of functions
4. Two-component system in Plants
5. Components and process ;
6. Examples of functions
7. Signalling **pathway**

12.6 SUGGESTED READINGS

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LESSON- 13

SIGNAL TRANSDUCTION AND GENE EXPRESSION

OBJECTIVES:

To learn signal transduction and gene expression is to understand how cells receive external cues (reception), convert them into internal messages (transduction), and trigger specific responses, often by altering gene activity (gene expression) to control growth, differentiation, metabolism, or survival, linking environmental changes to cellular function and organismal health.

STRUCTURE OF THE LESSON:

13.1 INTRODUCTION

Signal transduction is a process where external signals are converted into a cellular response, which often includes the regulation of gene expression. This conversion is achieved through a cascade of molecular events, starting when a signal (like a hormone) binds to a cell surface receptor, leading to changes in transcription factors that ultimately turn genes on or off. For example, a signal can activate a protein kinase, which then phosphorylates a transcription factor, allowing it to enter the nucleus and initiate the transcription of specific genes into messenger RNA (mRNA).

Initiation: A signaling molecule, or ligand, binds to a receptor protein on the cell surface.

Transduction: The binding event triggers a cascade of events inside the cell, often involving enzymes like protein kinases.

Relay: These events relay the signal from the cell surface to a variety of intracellular targets, sometimes using "second messengers" like cyclic AMP.

Cellular Response: The ultimate cellular response can

Signal transduction is the process by which an extracellular signal is converted into a cellular response, frequently involving the **regulation of gene expression**. Signaling pathways modify the activity of transcription factors (TFs), ultimately leading to changes in the genes that are transcribed into proteins.

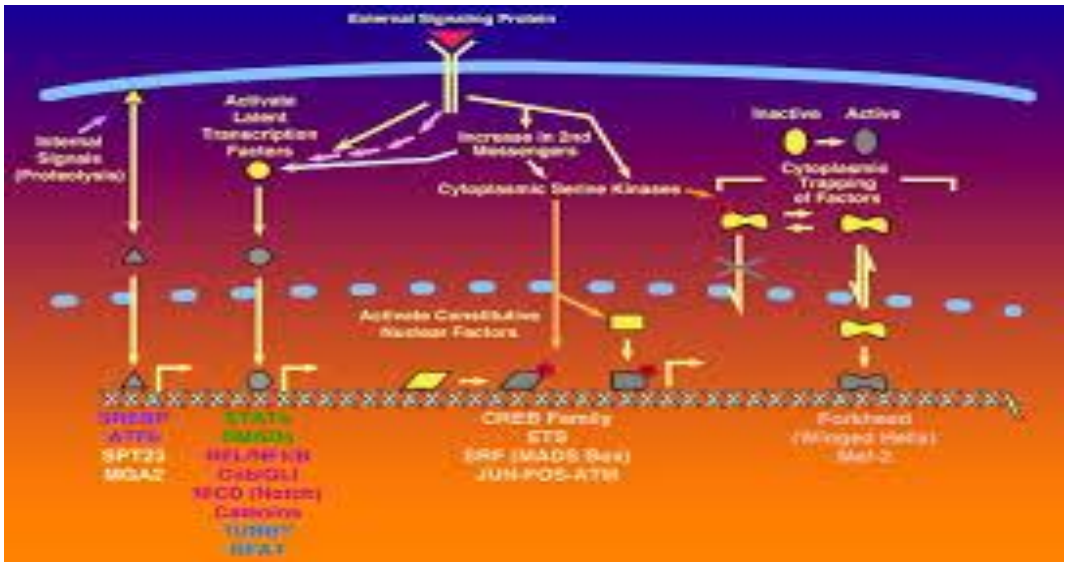
13.2 STAGES OF SIGNAL TRANSDUCTION

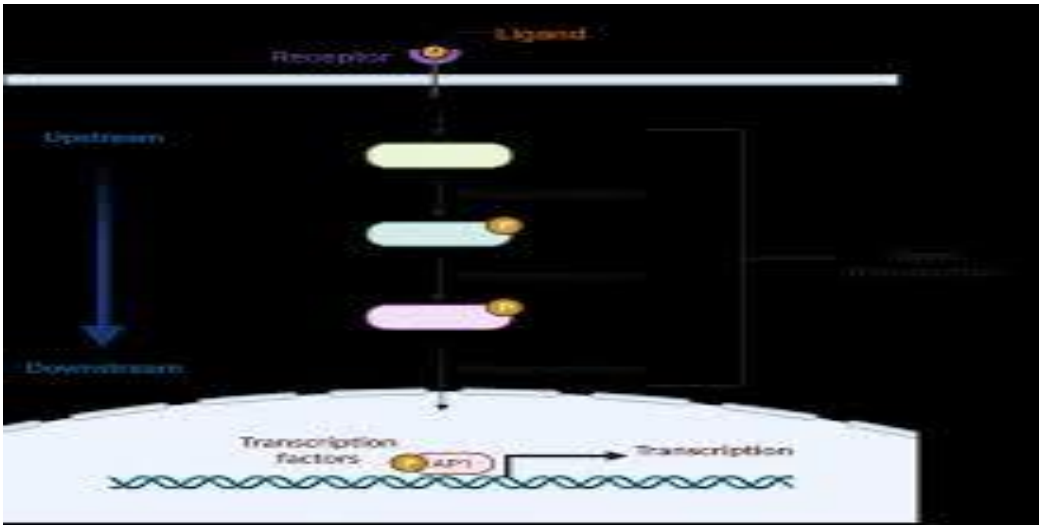
Signal transduction generally involves three main stages:

1. **Reception:** A signaling molecule (ligand), such as a hormone or growth factor, binds to a specific receptor protein on the cell surface or inside the cell.

2. **Transduction:** The binding of the ligand changes the receptor's shape, initiating a series of molecular events (a signaling cascade) within the cell. These events often involve the production of "second messengers" (e.g., cyclic AMP, calcium ions) and a cascade of protein phosphorylations carried out by protein kinases.

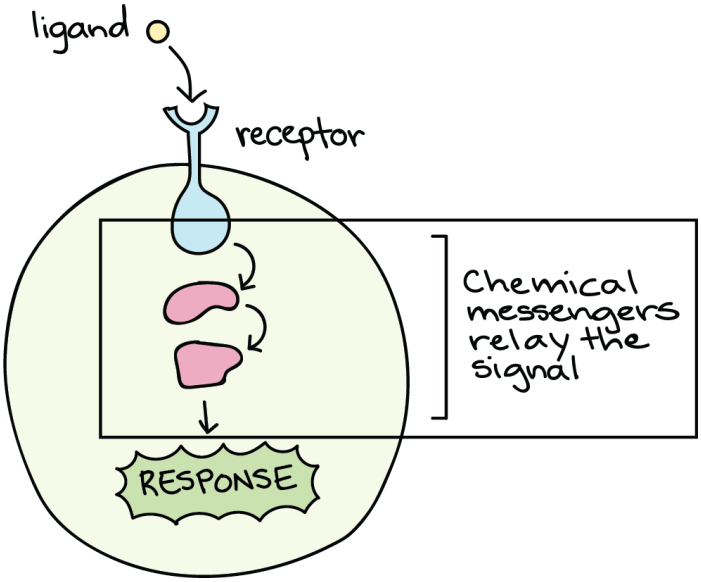
3. **Response:** The final activated molecules in the cascade trigger a specific cellular response, which often includes changes in gene expression.





Intracellular receptors

Intracellular receptors, which bind their ligand inside of the cell and directly activate genes, the answer may be yes. In most cases, though, the answer is no—not by a long shot! For receptors located on the cell membrane, the signal must be passed on through other molecules in the cell, in a sort of cellular game of "telephone."



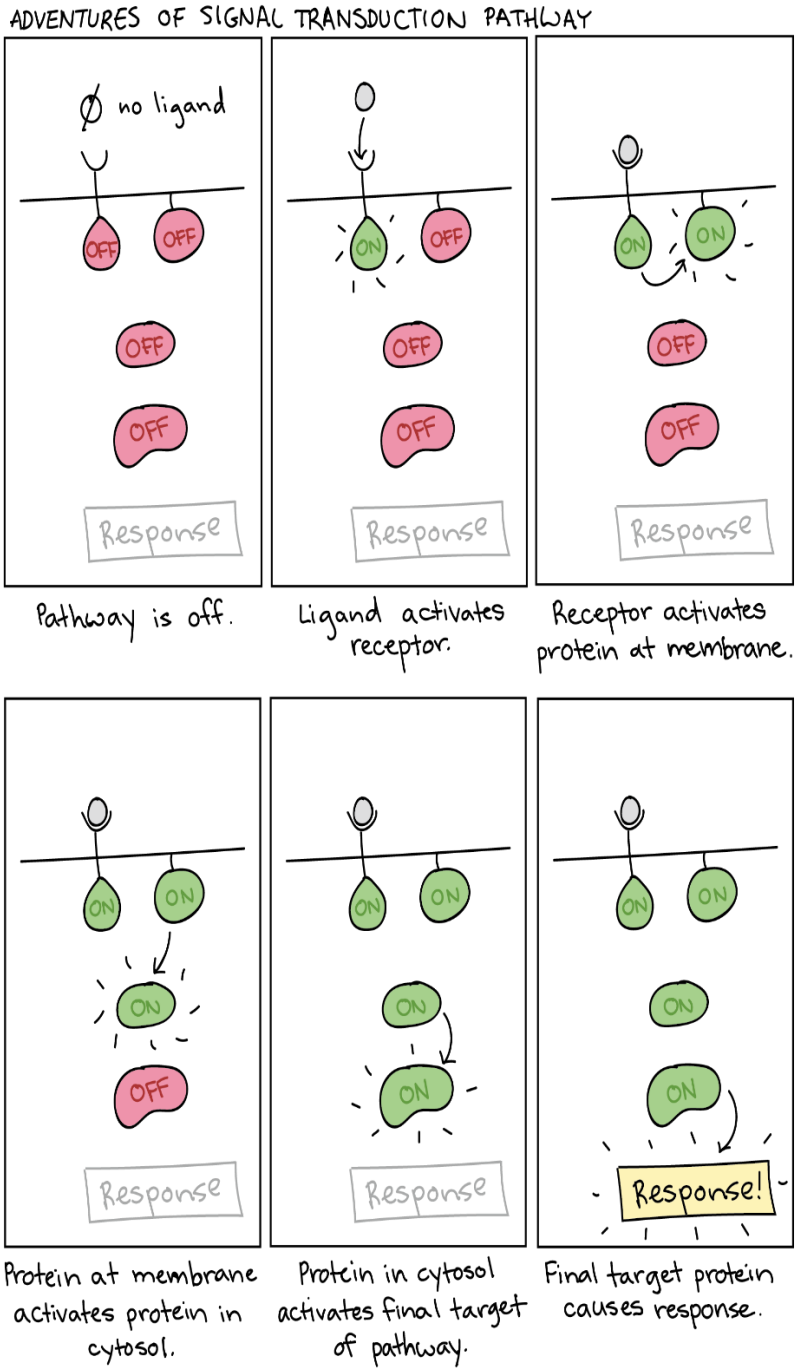
The chains of molecules that relay signals inside a cell are known as **intracellular signal transduction pathways**. Here, we'll look at the general characteristics of intracellular signal transduction pathways, as well as some relay mechanisms commonly used in these pathways.

Binding initiates a signaling pathway

When a ligand binds to a cell-surface receptor, the receptor's intracellular domain (part inside the cell) changes in some way. Generally, it takes on a new shape, which may make it active as an enzyme or let it bind other molecules.

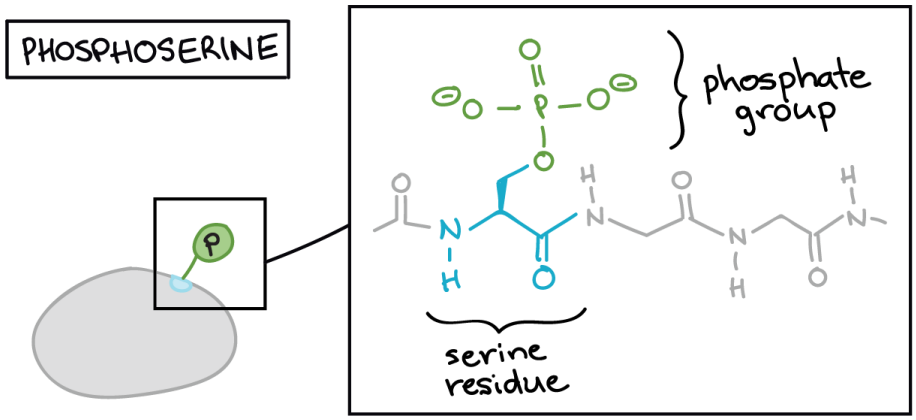
The change in the receptor sets off a series of signaling events. For instance, the receptor may turn on another signaling molecule inside of the cell, which in turn activates its own target. This chain reaction can eventually lead to a change in the cell's behavior or characteristics, as shown in the cartoon below. Because of the directional flow of information, the term **upstream** is often used to describe molecules and events that come earlier in the relay chain, while **downstream** may be used to describe those that come later (relative to a particular molecule of interest). For instance, in the diagram, the receptor is downstream of the ligand but upstream of the the proteins in the cytosol. Many signal transduction pathways amplify the initial signal, so that one molecule of ligand can lead to the activation of many molecules of a downstream target.

The molecules that relay a signal are often proteins. However, non-protein molecules like ions and phospholipids can also play important roles.



Phosphorylation

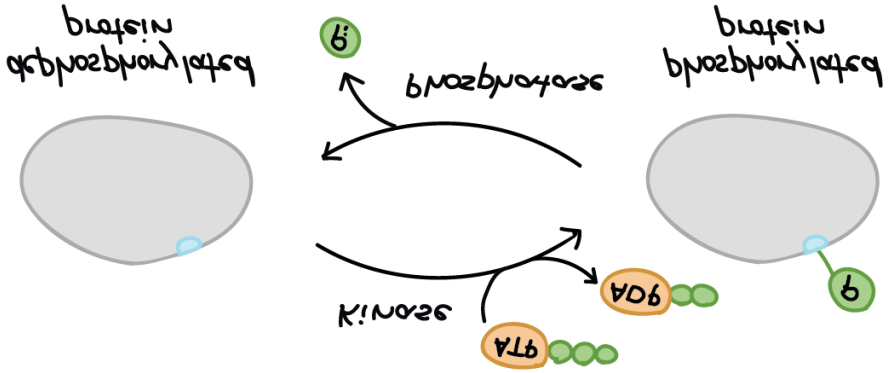
The cartoon above features a bunch of blobs (signaling molecules) labeled as “on” or “off.” What does it actually mean for a blob to be on or off? Proteins can be activated or inactivated in a variety of ways. However, one of the most common tricks for altering protein activity is the addition of a phosphate group to one or more sites on the protein, a process called **phosphorylation**.



Phosphate groups can't be attached to just any part of a protein. Instead, they are typically linked to one of the three amino acids that have hydroxyl (-OH) groups in their side chains: tyrosine, threonine, and serine. The transfer of the phosphate group is catalyzed by an enzyme called a **kinase**, and cells contain many different kinases that phosphorylate different targets.

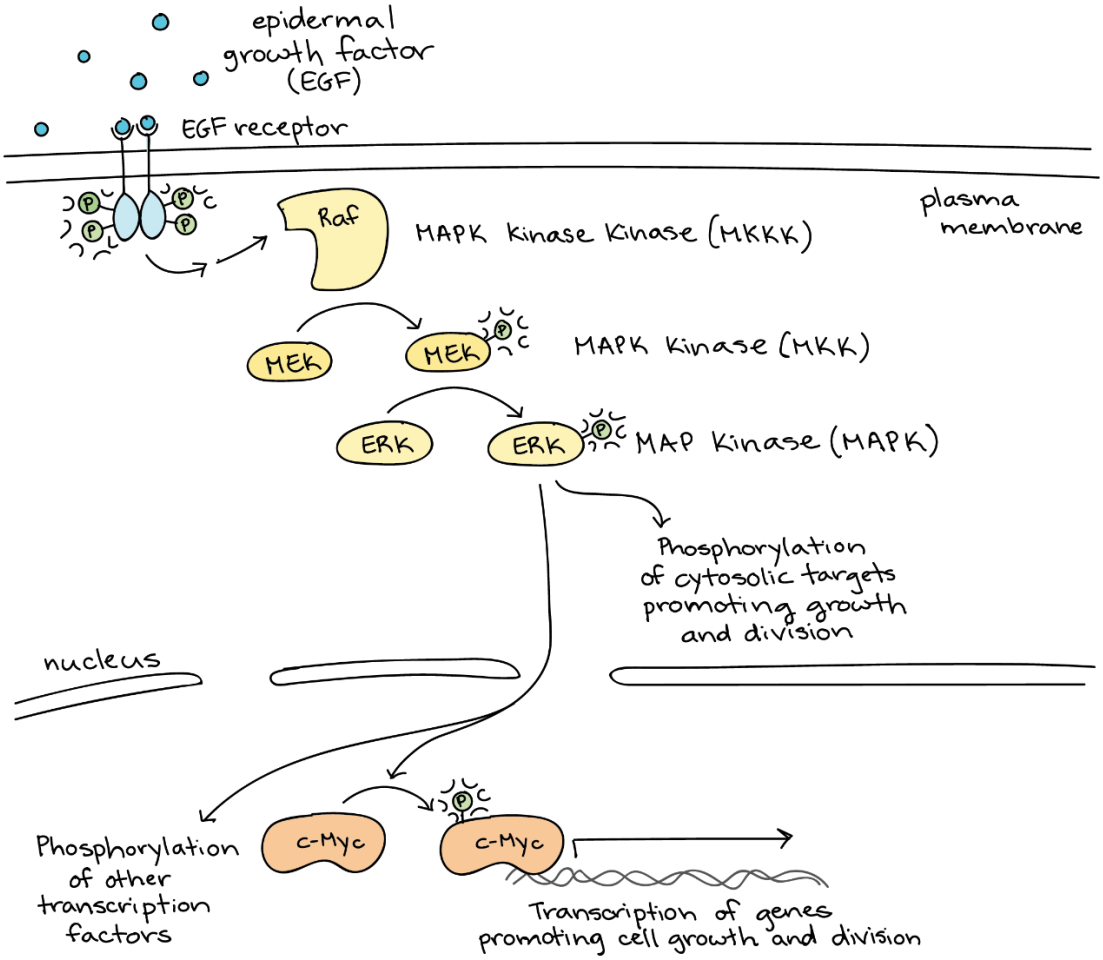
Phosphorylation often acts as a switch, but its effects vary among proteins. Sometimes, phosphorylation will make a protein more active (for instance, increasing catalysis or letting it bind to a partner). In other cases, phosphorylation may inactivate the protein or cause it to be broken down.

In general, phosphorylation isn't permanent. To flip proteins back into their non-phosphorylated state, cells have enzymes called **phosphatases**, which remove a phosphate group from their targets.



Phosphorylation example: MAPK signaling cascade

To get a better sense of how phosphorylation works, let's examine a real-life example of a signaling pathway that uses this technique: growth factor signaling. Specifically, we'll look at part of the epidermal growth factor (EGF) pathway that acts through a series of kinases to produce a cellular response.
This diagram shows part of the epidermal growth factor signaling pathway:



Phosphorylation (marked as a P) is important at many stages of this pathway.

- When growth factor ligands bind to their receptors, the receptors pair up and act as kinases, attaching phosphate groups to one another's intracellular tails. Read more in the article on receptors and ligands.
- The activated receptors trigger a series of events (skipped here because they don't involve phosphorylation). These events activate the kinase Raf.
- Active Raf phosphorylates and activates MEK, which phosphorylates and activates the ERKs.
- The ERKs phosphorylate and activate a variety of target molecules. These include transcription factors, like c-Myc, as well as cytoplasmic targets. The activated targets promote cell growth and division.

Together, Raf, MEK, and the ERKs make up a three-tiered kinase signaling pathway called a **mitogen-activated protein kinase** (**MAPK**) cascade. (A *mitogen* is a signal that causes cells to undergo *mitosis*, or divide.) Because they play a central role in promoting cell division, the genes encoding the growth factor receptor, Raf, and c-Myc are all proto-oncogenes, meaning that overactive forms of these proteins are associated with cancer].

MAP kinase signaling pathways are widespread in biology: they are found in a wide range of organisms, from humans to yeast to plants. The similarity of MAPK cascades in diverse organisms suggests that this pathway emerged early in the evolutionary history of life and was already present in a common ancestor of modern-day animals, plants, and fungi.

Second messengers

Although proteins are important in signal transduction pathways, other types of molecules can participate as well. Many pathways involve **second messengers**, small, non-protein molecules that pass along a signal initiated by the binding of a ligand (the "first messenger") to its receptor.
Second messengers include Ca2+ ions; cyclic AMP (cAMP), a derivative of ATP; and inositol phosphates, which are made from phospholipids.

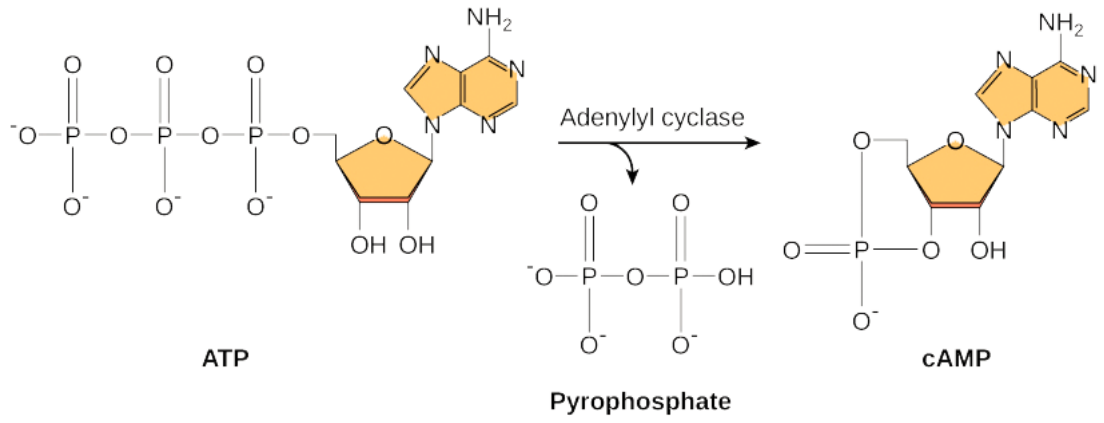
Calcium ions

Calcium ions are a widely used type of second messenger. In most cells, the concentration of calcium ions Ca2+ in the cytosol is very low, as ion pumps in the plasma membrane continually work to remove it. For signaling purposes, Ca2+ may be stored in compartments such as the endoplasmic reticulum.

In pathways that use calcium ions as a second messenger, upstream signaling events release a ligand that binds to and opens ligand-gated calcium ion channels. These channels open and allow the higher levels of Ca2+ that are present outside the cell (or in intracellular storage compartments) to flow into the cytoplasm, raising the concentration of cytoplasmic Ca2+
How does the released Ca2+ help pass along the signal? Some proteins in the cell have binding sites for Ca2+ ions, and the released ions attach to these proteins and change their shape (and thus, their activity). The proteins present and the response produced are different in different types of cells. For instance,Ca2+ signaling in the β-cells of the pancreas leads to the release of insulin, whileCa2+ signaling in muscle cells leads to muscle contraction.

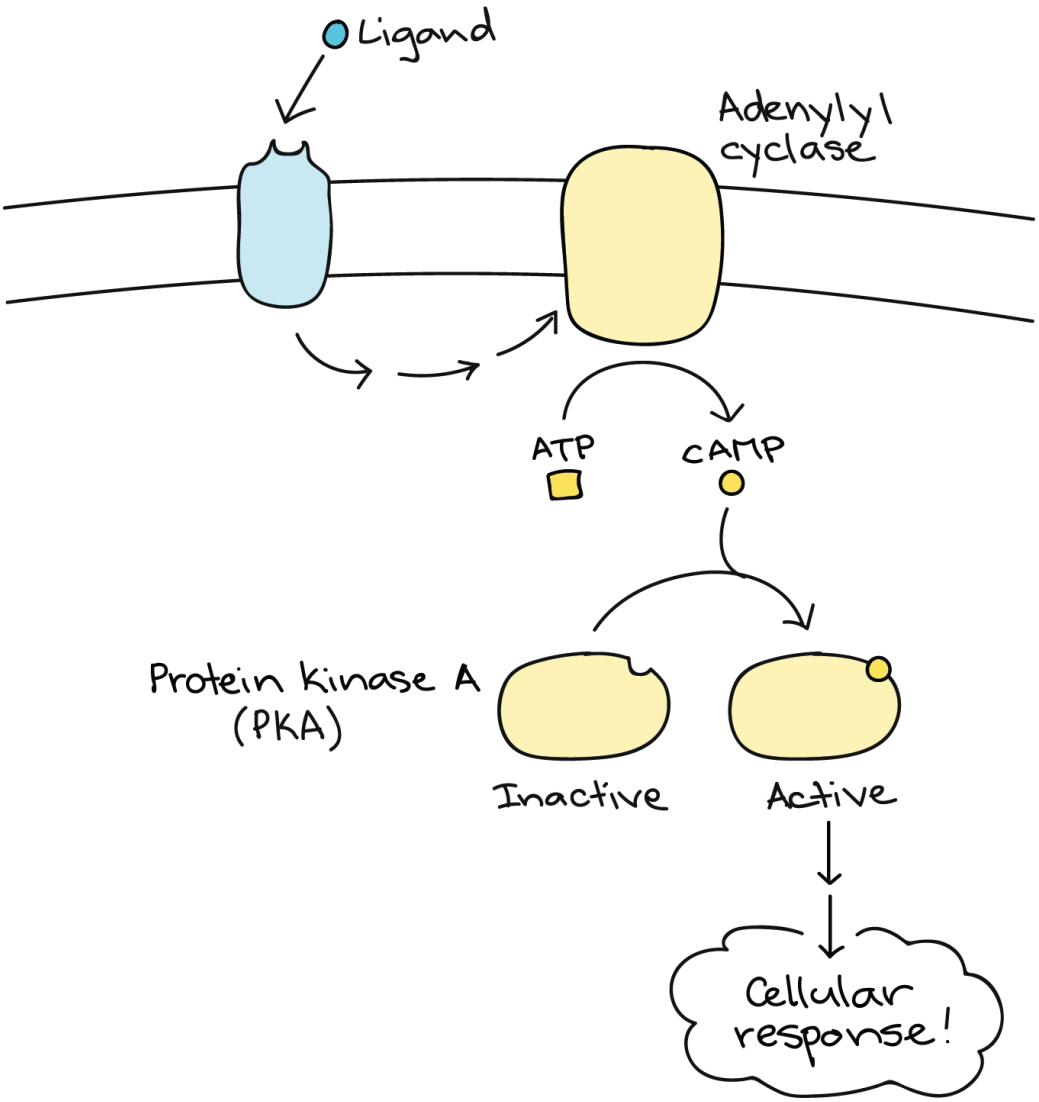
Cyclic AMP (cAMP)

Another second messenger used in many different cell types is **cyclic adenosine monophosphate** (**cyclic AMP** or **cAMP**), a small molecule made from ATP. In response to signals, an enzyme called **adenylyl cyclase** converts ATP into cAMP, removing two phosphates and linking the remaining phosphate to the sugar in a ring shape.



Once generated, cAMP can activate an enzyme called **protein kinase A** (**PKA**), enabling it to phosphorylate its targets and pass along the signal. Protein kinase A is found in a variety of

types of cells, and it has different target proteins in each. This allows the same cAMP second messenger to produce different responses in different contexts.

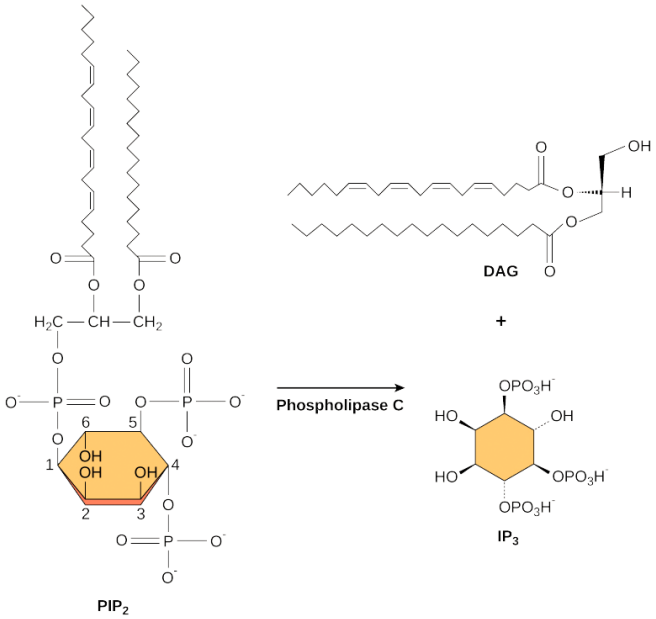


cAMP signaling is turned off by enzymes called **phosphodiesterases**, which break the ring of cAMP and turn it into adenosine monophosphate (AMP).

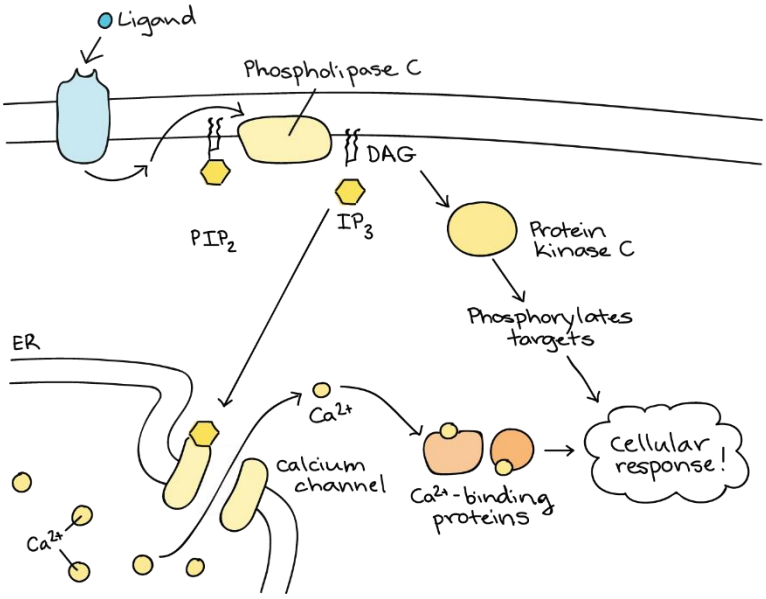
Inositol phosphates

Although we usually think of plasma membrane phospholipids as structural components of the cell, they can also be important participants in signaling. Phospholipids called **phosphatidylinositols** can be phosphorylated and snipped in half, releasing two fragments that both act as second messengers.

One lipid in this group that's particularly important in signaling is called PIP2. In response to a signal, an enzyme called phospholipase C cleaves (chopsPIP2 into two fragments, DAG and IP3. These fragments made can both act as second messengers.

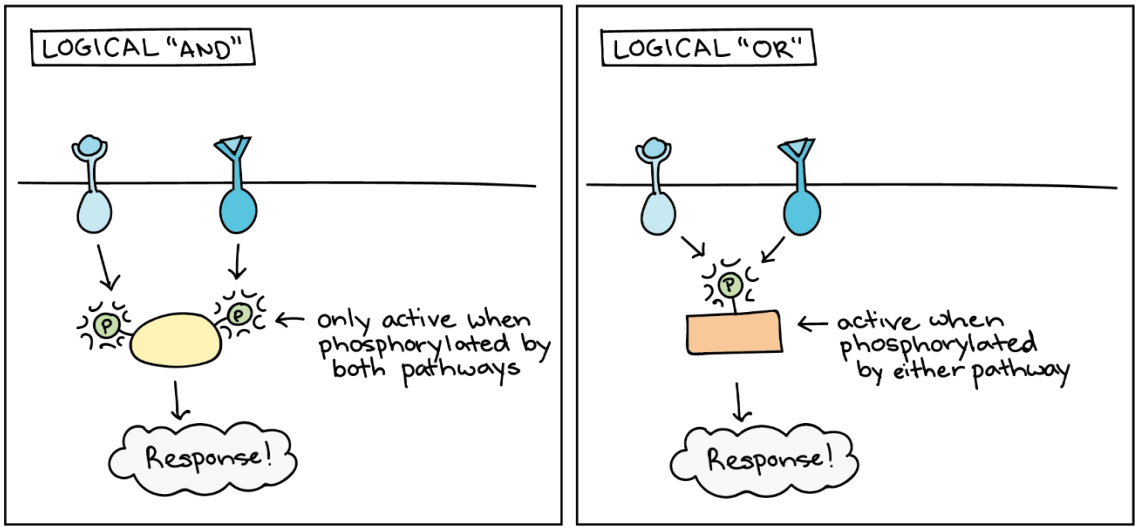


DAG stays in the plasma membrane and can activate a target called protein kinase C (PKC), allowing it to phosphorylate its own targets. \[\text{IP}_3\] diffuses into the cytoplasm and can bind to ligand-gated calcium channels in the endoplasmic reticulum, releasing that continues the signal cascade.

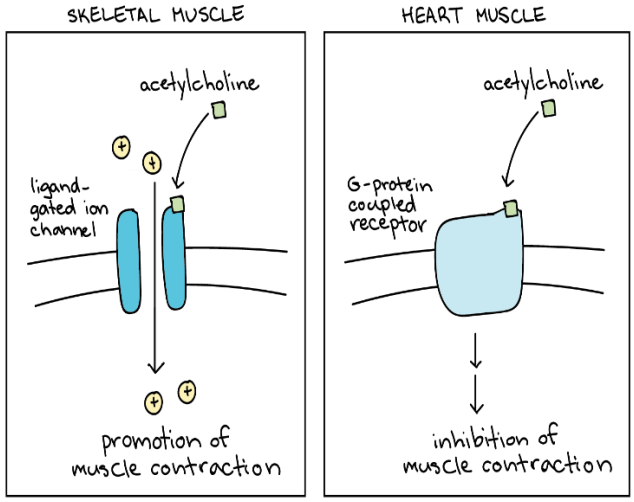


Signaling pathways can get very complicated very quickly. For instance, the full version of the epidermal growth factor signaling pathway we saw earlier looks like a huge hairball and takes up an entire poster if you try to draw it out! You can see this for yourself in Sal's video on the MAPK pathway.

This complexity arises because pathways can, and often do, interact with other pathways. When pathways interact, they basically allow the cell to perform logic operations and "calculate" the best response to multiple sources of information. For instance, signals from two different pathways may be needed to activate a response, which is like a logical "AND." Alternatively, *either* of two pathways may trigger the same response, which is like a logical "OR."



Another source of complexity in signaling is that the same signaling molecule may produce different results depending on what molecules are already present in the cell\[^3\]. For example, the ligand acetylcholine causes opposite effects in skeletal and heart muscle because these cell types produce different kinds of acetylcholine receptors that trigger different pathways.



These are just a few examples of the complexities that make signaling pathways challenging, but also fascinating, to study. Cell-cell signaling pathways, especially the epidermal growth factor pathway we saw earlier, are a focus of study for researchers developing new drugs against cancer

13.3 LINKING TO GENE EXPRESSION

The connection between signal transduction and gene expression primarily occurs in the nucleus. Key mechanisms include:

- **Transcription Factor Modulation:** Signaling pathways directly affect the activity, localization, and DNA-binding ability of transcription factors. For example, a protein kinase A (PKA) activated by cAMP in the cytoplasm can move into the nucleus and phosphorylate the transcription factor CREB (cAMP-response element-binding protein), which then binds to DNA and activates target genes.

- **Intracellular Receptors:** Lipid-soluble hormones (like steroids) can diffuse across the plasma membrane and bind to intracellular receptors in the cytoplasm or nucleus. Once activated, the hormone-receptor complex acts directly as a transcription factor, binding to specific DNA sequences to regulate gene expression.

- **Kinase Cascades:** Pathways like the Mitogen-Activated Protein Kinase (MAPK) cascade involve a series of kinases that phosphorylate each other. The final kinase in the sequence can translocate to the nucleus and phosphorylate transcription factors to induce gene expression, particularly those genes involved in cell proliferation and differentiation.

- **Epigenetic Modification:** Signal transduction pathways can also influence gene expression by regulating proteins that modify chromatin structure (e.g., histone modifications), making genes more or less accessible for transcription.

Ultimately, these molecular changes lead to the selective transcription of specific genes, allowing the cell to synthesize new proteins and alter its function or behavior in response to the initial external signal. Disruptions in these intricate pathways can lead to diseases like cancer.

13.4 GENE EXPRESSION

Gene exression involves with the following stages

Transcription Factor Activation: A key outcome of signal transduction is the activation of transcription factors, proteins that control gene expression.

Nuclear Entry: A signal can cause a transcription factor to move into the nucleus.

DNA Binding: Once in the nucleus, the transcription factor binds to specific DNA sequences near a gene.

Transcription: This binding event recruits the machinery needed for transcription, allowing RNA polymerase to create a messenger RNA (mRNA) copy of the gene.

Protein Synthesis: The mRNA then exits the nucleus and is used by ribosomes to synthesize new proteins, which carry out the cell's response.

13.5 SIGNAL TRANSDUCTION PATHWAY

Signal transduction occurs through the transfer of phosphoryl groups from adenosine triphosphate (ATP) to a specific histidine residue in the histidine kinases (HK). This is an autophosphorylation reaction. Subsequently the histidine kinase catalyzes the transfer of the phosphate group on the phosphorylated histidine residues to an aspartic acid residue on the response regulator (RR). Phosphorylation causes the response regulator’s conformation to change, usually activating an attached output domain, which then leads to the stimulation (or repression) of expression of target genes. The level of phosphorylation of the response regulator controls its activity. Some HK are bifunctional, catalysing both the phosphorylation and dephosphorylation of their cognate RR. The input stimuli can regulate either the kinase or phosphatase activity of the bifunctional HK.

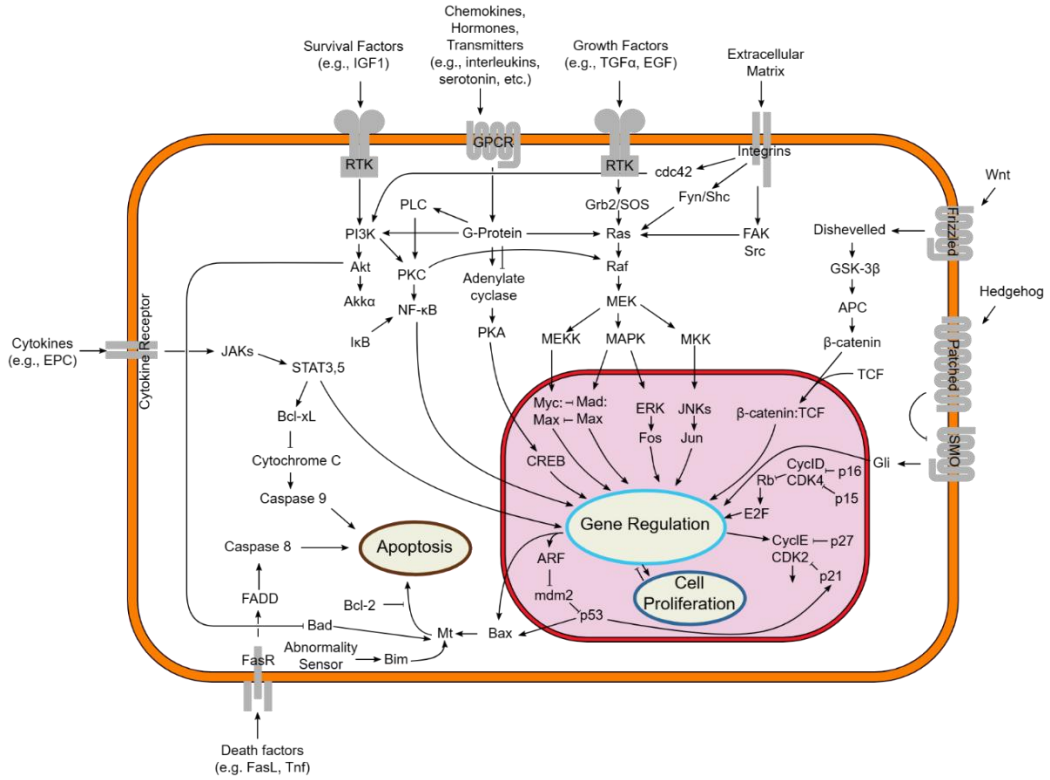


Figure: **Signal transduction**: An overview of major signal transduction pathways.

Two-component signal transduction systems enable bacteria to sense, respond and adapt to a wide range of environments, stressors and growth conditions. Some bacteria can contain as many as 200 two-component systems that need tight regulation to prevent unwanted cross-talk. These pathways have been adapted to respond to a wide variety of stimuli, including nutrients, cellular redox state, changes in osmolarity, quorum signals, antibiotics, temperature, chemoattractants, pH and more. In E. coli the EnvZ/OmpR osmoregulation system controls the differential expression of the outer membrane porin proteins OmpF and OmpC. The KdpD sensor kinase proteins regulate the kdpFABC operon responsible for potassium transport in bacteria including E. coli and Clostridium acetobutylicum. The N-terminal domain of this protein forms part of the cytoplasmic region of the protein, which may be the sensor domain responsible for sensing turgor pressure.

13.6 SYSTEM VARIANTS IN SIGNAL TRANSDUCTION

A variant of the two-component system is the phospho-relay system. Here a hybrid HK autophosphorylates and then transfers the phosphoryl group to an internal receiver domain, rather than to a separate RR protein. The phosphoryl group is then shuttled to histidine phosphotransferase (HPT) and subsequently to a terminal RR, which can evoke the desired response.

Signal transducing histidine kinases are the key elements in two-component signal transduction systems. Examples of histidine kinases are EnvZ, which plays a central role in osmoregulation, and CheA, which plays a central role in the chemotaxis system. Histidine kinases usually have an N-terminal ligand-binding domain and a C-terminal kinase domain, but other domains may also be present. The kinase domain is responsible for the

autophosphorylation of the histidine with ATP, the phosphotransfer from the kinase to an aspartate of the response regulator, and (with bifunctional enzymes) the phosphotransfer from aspartyl phosphate back to ADP or to water. The kinase core has a unique fold, distinct from that of the Ser/Thr/Tyr kinase superfamily.

HKs can be roughly divided into two classes: orthodox and hybrid kinases. Most orthodox HKs, typified by the Escherichia coli EnvZ protein, function as periplasmic membrane receptors and have a signal peptide and transmembrane segment(s) that separate the protein into a periplasmic N-terminal sensing domain and a highly conserved cytoplasmic C-terminal kinase core. Members of this family, however, have an integral membrane sensor domain. Not all orthodox kinases are membrane bound, e.g., the nitrogen regulatory kinase NtrB (GlnL) is a soluble cytoplasmic HK. Hybrid kinases contain multiple phosphodonor and phosphoacceptor sites and use multi-step phospho-relay schemes instead of promoting a single phosphoryl transfer. In addition to the sensor domain and kinase core, they contain a CheY-like receiver domain and a His-containing phosphotransfer (HPt) domain.

13.7 SUMMARY

Signal transduction is a cell's process of converting an external signal into an internal response, often a change in gene expression. It begins when a signaling molecule binds to a cell receptor, triggering a cascade of intracellular events, such as the activation of proteins and second messengers, that ultimately alter the activity of transcription factors. Gene expression is the process by which the information from a gene is used in the synthesis of a functional gene product, such as a protein.

13.8 MODEL QUESTIONS

1. Describe different stages of Signal Transduction
2. Describe the Linking to Gene Expression
3. Describe Gene expression
4. Describe Signal transduction pathway
5. Describe System Variants in signal transduction

13.9 Suggested Readings

1. Devline and Witham, 1986. Plant Physiology.CBSPubis.and Distributors. New Delhi.
2. Hopkins, W.G. 1995. Introduction to Plant Physiology, John Wiley & Sons. Inc., New York, USA.
3. Moore, T.C. 1989. Biochemistry and Physiology of Plant Hormones. Springer Verlag, New York, USA.
4. Singhal *et al*. 1999. Concepts in Photobiology. Photosynthesis and Photo-morphogenesis, Narosa Pub. House. New Delhi.
5. Taiz and Zeiger, 1998. Plant Physiology. Sinauer Associates Inc., Publishers, Sunderland.
6. Salisbury F.B & C. W. Ross, 1992. Plant Physiology, 4th Edition. Wadsworth Publishing Co., Belmount, California.

Prof A.Amrutha Valli

LESSON- 14
PLANT STRESS PHYSIOLOGY

OBJECTIVE:

In this lesson, definition for stress, the effects of water deficits or drought on plants and how plants respond to water stress, heat-shock proteins, and hypersensitive reaction and systemic acquired resistance developed in response to biotic stress in plants are discussed.

STRUCTURE OF THE LESSON:

14.1 INTRODUCTION

Plants, being stationary organisms, are continuously under the influence of different environmental conditions that negatively influence their growth, development, and productivity. These undesirable conditions are collectively classified as stress, either abiotic (non-biological factors such as drought, salinity, temperature, flooding, and heavy metals) or biotic (due to living organisms like pathogens, herbivores, and competing plants). Plant stress results in intricate physiological, biochemical, and molecular responses for the purpose of survival and adaptation. These include stomatal closure, osmolyte accumulation (proline), activation of antioxidant defense mechanisms, and expression of stress-responsive genes and proteins like heat shock proteins (HSPs) and transcription factors (e.g., DREB, WRKY). Signal transduction mechanisms with calcium ions, protein kinases, and plant hormones such as abscisic acid (ABA), jasmonic acid, and salicylic acid are important in facilitating these responses. Epigenetic modifications and stress memory at the molecular level are becoming increasingly well known. Knowledge on plant response to stress is of prime importance in breeding climate-resistant crops through recent advances in breeding, genetic engineering, and biotechnology. For graduate students, learning plant stress physiology lays the groundwork for solving key agricultural issues and is part of achieving sustainable food production under climatic variability.

14.1.1 Stress:

Any strain or interference that disturbs the functioning of an organism can be defined as stress.

Plant Stress: An external factor that exerts a disadvantageous influence on the plant causing an impairment or reduction in metabolism or development. Plant stress physiology deals with a) the characterization of stress induced changes in plants b) finding the most effective ways to avoid or tolerate stresses.

The study of plant responses to environmental stress has long been a central theme for plant environmental physiologists and physiological ecologists. How plants respond to stress helps to explain their geographic distribution and their performance along environmental gradients. Because stress invariably leads to reduced productivity, **stress responses** are also important to agricultural scientists. Understanding stress responses is essential in attempts to breed ress-resistant cultivars that can withstand drought, and other yield-limiting conditions. Finally, because stressful conditions cause perturbations in the way a plant functions, they provide the plant physiologist with another very useful tool for the study of basic physiology and biochemistry.

Some of the stresses thatplants encounter in their environment. The principal topics to be addressed include
• the basic concepts of plant stress, acclimation, and adaptation,
• the light-dependent inhibition of photosynthesis through a process called photoinhibition,
• the effects of water deficits on stomatal conductance,
• the effects of high- and low-temperature stress on plant survival,
• the challenge of freezing stress on plant survival, and
• the responses of plants to biotic stress due to infestations by insects and disease.

Because life is an endergonic process, that is 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. This means that individual organisms are not closed systems but are open systems relative to their surrounding environment. This 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. Such energy flow ensures that living biological organisms are never at equilibrium with their environment, that is, _G is never equal to zero, but remain in a steady-state condition far from equilibrium The maintenance of such a steady-state results in a meta-stable condition called **homeostasis**.As a consequence, all life forms may be considered transient energy storage devices with finite but varyinglifetimes (Figure 14.2.1).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 (Figure 13.3). 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. Since the extent of a stress can be quantified by assessing the difference in these measurements under optimal versus suboptimal conditions, the basis of stress physiology is **comparative**

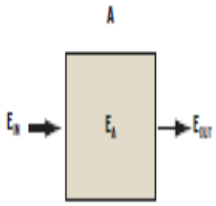


FIGURE 13.2 Biological life forms as energy-storing devices. A is any biological life form. EIN (thick arrow) is the energy flowing from the surrounding environment into the biological organism, A. EOUT (thin arrow) is the energy flowing out of the biological organism back into the environment. The thicknesses of the arrows indicate the differences in the relative flux of energy flowing in and out of a living organism. EA is the steady-state energy stored or trapped by a living organism. According to the First Law of thermodynamics (Chapter 5), EIN + EA + EOUT = 1. Thus, EA = EIN − EOUT.

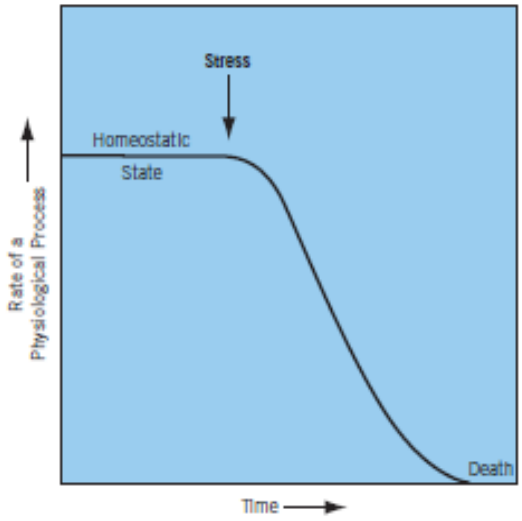


FIGURE 13.3 The effects of environmental stress on plant homeostasis. Under some optimal environmental condition,a plant is in homeostasis as indicated by a constant rate of some important physiological process over time. Upon the imposition of an external stress, the rate of this physiological process decreases rapidly. It is fatal for some plants that are unable to adjust to an imposed stress and can not establish a new homeostatic state. Such plants are classified as susceptible to the stress.

physiology. However, plant species are highly variable with respect to their optimum environments and their susceptibility to extremes of, for example, irradiance,temperature, and water potential. Is stress a function of the environment or the organism? For example, are the extreme environments encountered in deserts or arctic tundra stressful for plants that

normally thrive there? Are these environments stressful only to some species but not to others?

14.1.2 Classification of stress factors:

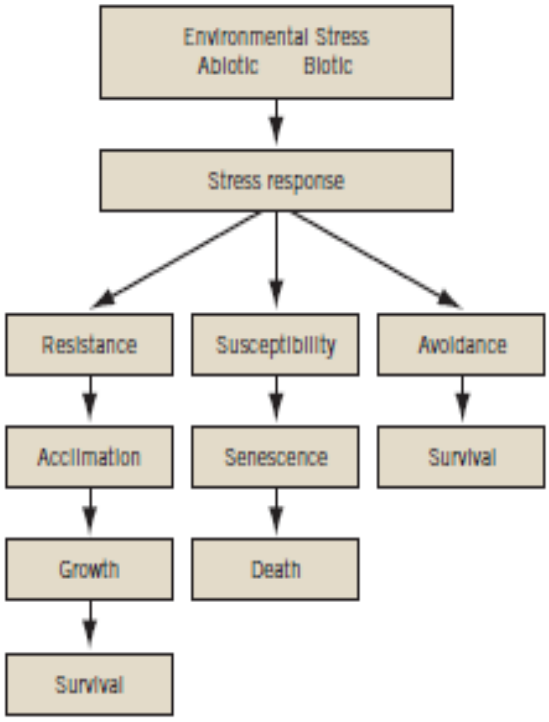


FIGURE 13.3 The effect of environmental stress on plant survival.

Plants generally undergoes two types of stress factors
1. Natural factors 2. Anthropogenic factors. The various stress factors are given in table 1.1.
Table 1.1 List of various stress factors on plants

Natural factors	**Anthropogenic factors**
extremes or abrupt changes	**pollution by human activity**
light intensity	herbicides
temperature	air, soil, water pollutants
water stress	acid rain
nutrient availability	enhanced UV-B radiation
Salt stress	
Cold stress	
Biotic stress	

14.2 Phases of stress:

Plant stress occurs in distinct phases, beginning with the perception phase (Figure 1.1), where plants detect environmental or physiological changes such as drought, salinity, heat, or pathogen attack through specialized receptors, often located in the cell membrane. This is followed by the signal transduction phase, in which the perceived stress triggers internal signaling cascades involving molecules like calcium ions, reactive oxygen species (ROS), and plant hormones such as abscisic acid (ABA). These signals activate various transcription factors and kinases that regulate stress-responsive genes. Next comes the response phase, where the plant initiates physiological, biochemical, and molecular adjustments such as stomatal closure, osmolyte accumulation (e.g., proline), antioxidant production, and expression of heat shock or late embryogenesis abundant (LEA) proteins to minimize damage and maintain cellular function. Depending on the intensity and duration of the stress, the plant enters either a tolerance phase, successfully adapting and surviving, or an injury phase, where prolonged exposure leads to irreversible damage like protein denaturation or membrane disruption. If the stress is alleviated in time, plants may undergo a recovery phase, during which they repair damaged tissues, re-establish metabolic balance, and resume normal growth. These phases collectively represent a dynamic and coordinated strategy through which plants cope with adverse conditions.

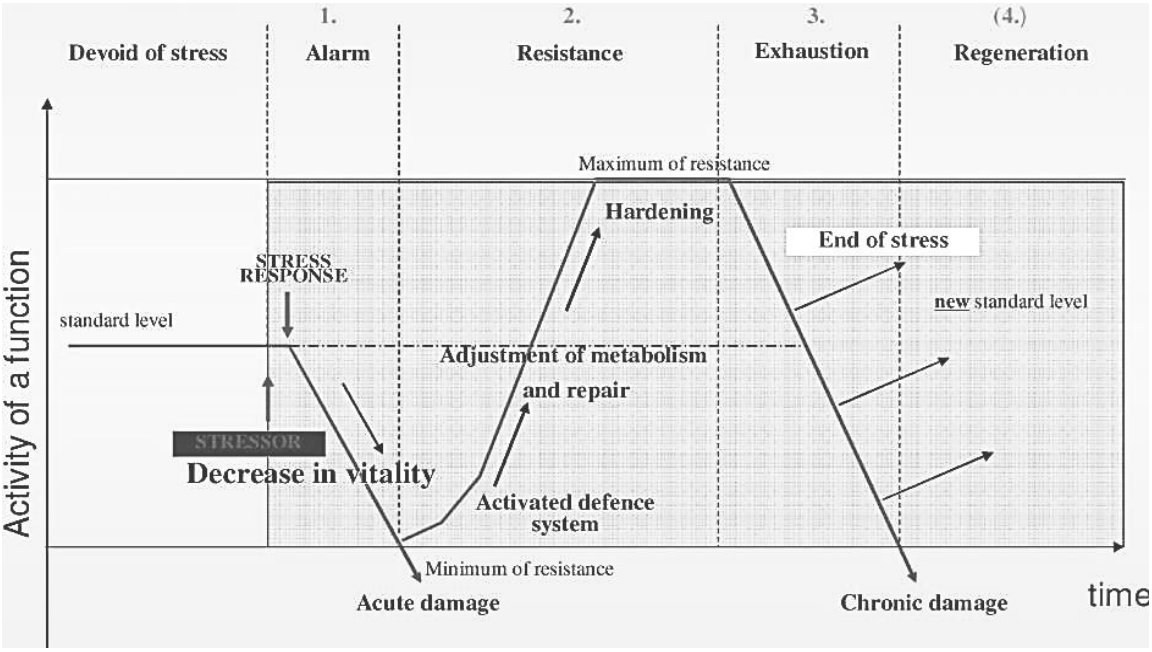


Figure 1.1 Various phase of plant stress

Plants, being sessile organisms, are continuously subjected to a variety of abiotic and biotic stresses. Their very survival depends upon the effective execution of a cascade of sophisticated physiological, biochemical, and morphological adjustments. These strategies for survival can be generally grouped into three distinct types 1. Stress avoiders 2. Stress adopters 3. Stress escapers.

1. Stress avoiders: These plants positively keeps its internal environment in an optimum state, thus avoiding the stress from penetrating and damaging its important tissues. It is usually a temporary or short-term mechanism. To avoid drought desert shrubs use a series of mechanisms to keep the tissue water potential high. They can have strong, deep root systems to tap groundwater or a waxy, thick cuticle and modified stomata to reduce water loss via

transpiration. For salt exclusion, some halophytes such as mangroves either exclude excess salts at the roots or secrete them through specialized leaf glands. In the same way, cold-avoiding plants like winter annuals avoid internal ice formation through super cooling, wherein cell sap's freezing point is reduced by solutes, or through the promotion of extracellular ice formation that dehydrates the cells but saves the protoplasm from freezing. These avoidance mechanisms are important to ensure cellular homeostasis under unfavourable external conditions.

2. Stress adopters: These plants have the capacity to survive and carry out its functions even under high stresses. The cells of the plant suffer from the stress, but the plant has strong systems to withstand the induced cellular injury. Drought-resistant plants, for example, the renowned resurrection plants, can survive severe drying by accumulating compatible solutes such as proline and sugars. This is called osmotic adjustment, which reduces the osmotic potential and supports turgor and enzymatic activity. In addition, such plants have highly effective antioxidant systems to detoxify the reactive oxygen species (ROS) formed during stress. Salt-tolerant halophytes are another category of salt adopters that sequester excess salts (Na^+ and Cl^-) in their large central vacuoles, thus keeping the cytoplasmic concentration of salt low and producing organic osmolytes to counteract the osmotic potential. For heat tolerance, plants quickly synthesize heat shock proteins (HSPs), acting as molecular chaperones to shield other proteins from denaturation. Lastly, certain plants can tolerate heavy metals by synthesizing phytochelatins, low-molecular-weight peptides that complex and sequester the toxic ions in the vacuole. These tolerance mechanisms are profound physiological and genetic adjustments permitting the plant to survive under conditions that are lethal to others.

3. Stress Escapers: Escaping stress is a phenological strategy, which differs from the cellular-level adaptations of avoidance and tolerance. It involves a plant completing its entire life cycle during a short, favorable period, thus "escaping" the prolonged, stressful season. This is a life-cycle-level adaptation, typically seen in annual plants with a rapid growth cycle. A classic example is the desert ephemerals, which lie dormant as seeds for years. Upon a rare rainfall event, they rapidly germinate, grow, flower, and produce new seeds before the soil dries out and the stressful dry season returns. The seeds then enter a state of dormancy, waiting for the next favorable opportunity. This strategy is a highly effective way for plants to survive in environments with unpredictable and short-lived periods of suitable conditions.

14.3 CROSS-TOLERANCE MECHANISM BETWEEN BIOTIC AND ABIOTIC STRESS

Various elements possibly involved in cross-tolerance between biotic and abiotic stress (Figure 1.2). Both biotic and abiotic stress have to be first sensed by the plant cell, and then the information is transduced to appropriate downstream-located pathway(s). Sensors as well as signal transducers might be shared by both types of stressors. Reactive oxygen species (ROS) and Ca^{++} are known among others to play a prominent role as transducers (messengers). Mitogen-activated protein kinases (MAPK) centrally positioned in Ca^{2+}-ROS crosstalk as well as in the signal output after exposure to a specific stress. The importance of ROS has repeatedly been described for both types of stresses too, and, therefore, ROS might represent crucial elements in the integration of both stresses during cross-tolerance. Plant hormone signalling is of utter importance for stress adaptation. While abscisic acid (ABA) is predominantly involved in abiotic stress adaptation, salicylic acid (SA) and jasmonate/ethylene (JA/ET) are more responsible for the plant’s reaction to biotic stress.

ABA signaling contributes positively to pre-invasion defense and is responsible for enhancing callose deposition. ABA presents a positive interaction with JA/ET signaling. The activation of SA signaling by pathogen challenge can attenuate ABA responses. ABA signaling negatively affects signals that trigger systemic acquired resistance, enhancing pathogen spread from the initial site of infection. The interaction of SA, JA, and ET signaling results in increased resistance to pathogens. Hormones, secondary metabolites, priming agents, and further chemicals located in the cytoplasm finally up-regulate transcription factors (TF), pathogenesis related (PR) and defense genes, heat shock protein (HSP) genes, and further genes involved in protection against stress and thus lead to the phenotypic expression known as cross-tolerance.

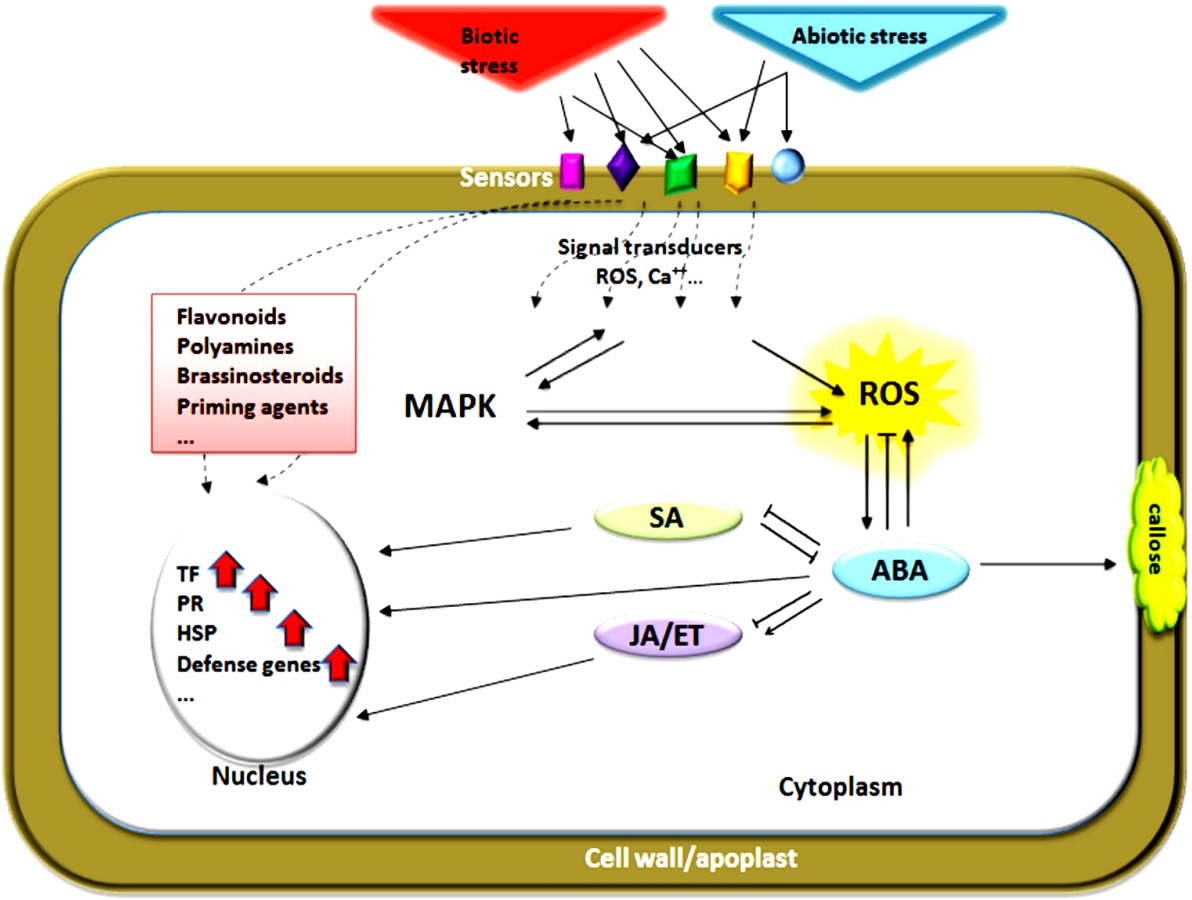


Figure 1.2 Elements possibly involved in cross-tolerance between biotic and abiotic stress

14.4 SUMMARY

Plants are subjected to two main types of stress—abiotic and biotic—that significantly influence plant growth, development, and productivity. Abiotic stresses arise from non-living elements of the environment such as drought, salinity, excessive heat, chilling, flooding, nutrient deficiency, toxicities of heavy elements, and excessive radiation. Abiotic stresses disrupt physiological and biochemical activities of plant cells like photosynthesis, water balance, and ion homeostasis and tend to invoke oxidative stress by ROS accumulation. Biotic stresses arise from living organisms such as fungi, bacteria, viruses, nematodes, insects, and weeds that injure plant tissues, compete for resources, or deliver diseases. Plants react to stresses by invoking elaborate defense and adaptation reactions such as synthesis of

protecting metabolites, stress proteins, and signaling molecules (e.g., abscisic acid, salicylic acid, and jasmonate) and deposition of structural barriers and antioxidant defenses. Cumulatively, these reactions allow plants to tolerate adverse conditions and ensure survival although often at the cost of reduced yield and output.

14.5 SELF ASSESSMENT QUESTIONS

1. Discuss the effects of abiotic stresses on plant physiology and metabolism.
2. Explain the role of ROS and antioxidant defense systems in plant stress tolerance.
3. Describe plant responses and defense mechanisms against biotic stresses.
4. Discuss the role of transcription factors in regulating stress-responsive genes.
5. Write a detailed note on phase of plant stress.
6. Describe the structural and biochemical defense mechanisms against pathogens.

14.6 SUGGESTED READINGS

1. Plant Stress Physiology: Sergey Shabala (CABI, 2012)
   A comprehensive book covering abiotic stresses like drought, salinity, temperature, and their physiological impact.
2. Abiotic Stress Adaptation in Plants: Physiological, Molecular and Genomic Foundation: Ashwani Pareek, Sudhir K. Sopory, Hans J. Bohnert, Govindjee (Springer, 2010).
3. Physiology and Molecular Biology of Stress Tolerance in Plants: K.V. Madhava Rao, A.S. Raghavendra, K. Janardhan Reddy (Springer, 2006).
4. Plant Stress Tolerance: Methods and Protocols: Aryadeep Roychoudhury & Durgesh Kumar Tripathi (Springer Protocols, 2021).

Dr K.Babu

LESSON -15

STRUCTURAL, PHYSIOLOGICAL, BIOCHEMICAL AND MOLECULAR RESPONSES OF PLANTS TO WATER STRESS

OBJECTIVE:

To understand the structural, physiological, biochemical, and molecular mechanisms by which plants perceive, respond, and adapt to water stress, thereby identifying strategies for improving drought tolerance.

STRUCTURE OF THE LESSON:

15.1 INTRODUCTION

Water plays a crucial role in determining the global distribution of plant species. Insufficient rainfall and tightly bound soil water often cause water deficiency in crops. Among various environmental factors, water stress is considered the most critical limitation to primary productivity in terrestrial ecosystems. Water stress occurs when the supply of water to plant roots becomes inadequate, leading to reduced transpiration rates. It is primarily induced by drought conditions or high soil salinity. Such stress disrupts agricultural systems, hampers food production, and can ultimately contribute to famine. Water deficit is one of the most widespread environmental challenges affecting plant growth and development. The impact of water stress varies with its severity, duration, and the developmental stage at which it occurs. Since water is essential for normal plant growth and development, both temporary and prolonged deficits can restrict the growth and distribution of natural vegetation as well as cultivated crops. Under water stress, plants undergo significant metabolic alterations, typically accompanied by reduced photosynthesis and growth. These physiological changes not only impair plant performance but also influence agricultural productivity, ecosystem stability, and, consequently, human society.

15.2 TYPES AND CAUSES OF WATER STRESS

Based on the availability of water to the plant, three types of water stress we can identify 1. Mild water deficit stress 2. Moderate water deficit stress 3. Severe water deficit stress.

1. Mild water deficit stress

In mild water stress, plant are subjected to minimum water stress. Mild water deficit stress causes an instant closure of stomata and a significant decrease in photosynthesis in C_3 plants. The plants tolerated mild stress, exhibited a high relative transpiration, low specific leaf weight and high carbon isotope discrimination in leaf and low leaf initial area. These characteristics resulted in keeping moisture content high in leaves to ensure an increase in leaf area, shoot dry matter, sugar, and starch content. Mild water stress results in increase in water use efficiency and degradation of sugar compared to the starch in leaf blades, leading to the increased accumulation of carbohydrates in leaf blades. It has been found that the mild stress causes a reduction in leaf area and leaf biomass per plant. This is due to the decreased production of new leaves, increased leaf shedding, and decreased average leaf size.

2 Moderate water deficit stress

Moderate water deficit stress does not severely affect the plants and the damage caused by this type of stress can be reversed. In common, plants subjected to moderate stress, a decrease in stomatal conductance and net photosynthetic rate is observed. Photo-inhibition is not found. Plants show a reduction in electron transport, which can be reversed by relieving the plants from the stress. In some plants moderate water stress is associated with root dehydration and leaf dehydration.

3 Severe water deficit stress

Severe water deficit stress on plants results in a reduction of root volume flux density and hydraulic conductivity resulting in an increase in apoplastic root flow pathway. Severe drought conditions result in progressive down regulation of metabolic processes, which leads to a decrease in RuBP content thus causing an inhibition in photosynthesis and carbon dioxide assimilation. Severe stress conditions results in decrease of root hydraulic conductivity. Severe water stress causes considerable membrane injuries in plants. This is accompanied by an increase in accumulation of proline, which is involved in alleviating membrane injuries in leaves. Severely water stressed plants show an increase in the expression of osmolality in sap and concentration of proline in leaves. Carbon dioxide assimilation and stomatal conductance rate decrease in such a way that intrinsic water use efficiency is increased. Severe water stress conditions showed a significant decrease in leaf relative water content, leaf biomass, whole plant biomass, photosynthetic rate, and water use efficiency. Stomatal index is found to increase in stress conditions but under severe stress, it does not increase.

15.3 EFFECT OF WATER STRESS ON PLANTS

Water stress applied to different types of plants shows different effects. If C_3 and C_4 plants are subjected to water deficit stress, it is observed that the effects of mild stress application do not differ for both kinds of plants. Moderate and high levels of stress were observed to cause more damage. C_3 plants experience a higher loss of water and chlorophyll compared to C_4 plants. This loss is also associated with oxidative damage, which is represented by an increase in malondialdehyde and hydrogen peroxide content. C_4 plants show a high content of enzymatic and non-enzymatic antioxidants. Some plants show an increased activity of catalase in roots and leaves. Root water content is also observed to be higher under stress conditions. Polyamine levels, also found to be increased in some plant

species. Leaf biomass reduces under prolonged water stress. Increase in lipid peroxidation and lignin content increase significantly with the increase in stress duration. In some cases leaf expansion, and development are significantly reduced but resume immediately after rewatering. As a result of drought, relative expansion rate and cell proliferation are also delayed resulting in loss of leaf area. Cell growth processes are very sensitive, even to mild drought stress. The cell reproduction rate is reduced in young meristematic leaves. Drought exposure has a minor effect on changes in the length of organic molecules. Seed yield per plant is highly affected by drought treatment. Water stress induces epigenetic state in chromatin in normal plants that may inherit to the progeny. This phenomenon refers to trans-generational epigenetic inheritance. Various morpho-physiological, biochemical and molecular responses of plants to water stress discussed in detail hereunder.

i) Decreased Leaf Area Is an Early Adaptive Response to Water Deficit

As the water content of the plant decreases, its cells shrink and the cell walls relax. This decrease in cell volume results in lower turgor pressure and the subsequent concentration of solutes in the cells. The plasma membrane becomes thicker and more compressed. Turgor reduction is the earliest and first significant biophysical effect of water stress, turgor-dependent activities such as leaf expansion and root elongation are the most sensitive to water deficits. Cell expansion is a turgor-driven process and is extremely sensitive to water deficit. Cell expansion is described by the relationship $GR = m(Yp - Y)$ where GR is growth rate, Yp is turgor, Y is the yield threshold, and m is the wall extensibility. This equation shows that a decrease in turgor causes a decrease in growth rate.

ii) Water deficit stimulates leaf abscission

The total leaf area of a plant does not remain constant after all the leaves have matured. If plants become water stressed after a substantial leaf area has developed, leaves will senesce and eventually fall off. Indeed, many drought-deciduous, desert plants drop all their leaves during a drought and sprout new ones after a rain. This cycle can occur two or more times in a single season. Abscission during water stress results largely from enhanced synthesis of and responsiveness to the endogenous plant hormone ethylene.

iii) Water deficit increases root growth

Inhibition of leaf expansion reduces the consumption of carbon and energy, and a greater proportion of the plant's assimilates can be distributed to the root system, where they can support further root growth. At the same time, the root apices in dry soil lose turgor. All these factors lead to a preferential root growth into the soil zones that remain moist. As water deficits progress, the upper layers of the soil usually dry first. Thus, plants commonly show a mainly shallow root system when all soil layers are wetted, and a loss of shallow roots and proliferation of deep roots as water in top layers of the soil is depleted. Deeper root growth into wet soil can be considered a second line of defense against drought. Enhanced root growth into moist soil zones during stress requires allocation of assimilates to the growing root tips. During water deficit, assimilates are directed to the fruits and away from the roots. For this reason the enhanced water uptake resulting from root growth is less pronounced in reproductive plants than in vegetative plants.

iv) Stomata close and Abscisic Acid

Stomatal closure can be considered a third line of defense against drought. Uptake and loss of water in guard cells changes their turgor and modulates stomatal opening and closing. A reduction in the solute content of the guard cells results in water loss and decreased turgor, causing the stomata to close. Solute loss from guard cells can be triggered by a decrease in the water content of the leaf, and abscisic acid (ABA). Abscisic acid is synthesized continuously at a low rate in mesophyll cells and tends to accumulate in the chloroplasts. When the mesophyll becomes mildly dehydrated, light stimulates proton uptake into the grana, making the stroma more alkaline. The increased alkalinity causes the weak acid ABA•H to dissociate into H+ and the ABA– anion. The concentration of ABA•H in the stroma is lowered below the concentration in the cytosol, and the concentration difference drives the passive diffusion of ABA•H across the chloroplast membrane. At the same time, the concentration of ABA– in the stroma increases, but the chloroplast membrane is almost impermeable to the anion (red arrows), which thus remains trapped (Figure 2.1). This process continues until the ABA•H concentrations in the stroma and the cytosol are equal. But as long as the stroma remains more alkaline, the total ABA concentration (ABA•H + ABA–) in the stroma greatly exceeds the concentration in the cytosol. The higher ABA concentrations resulting from the higher rates of ABA synthesis appear to enhance or prolong the initial closing effect of the stored ABA. Stomatal conductance is often much more closely related to soil water status than to leaf water status, and the only plant part that can be directly affected by soil water status is the root system.

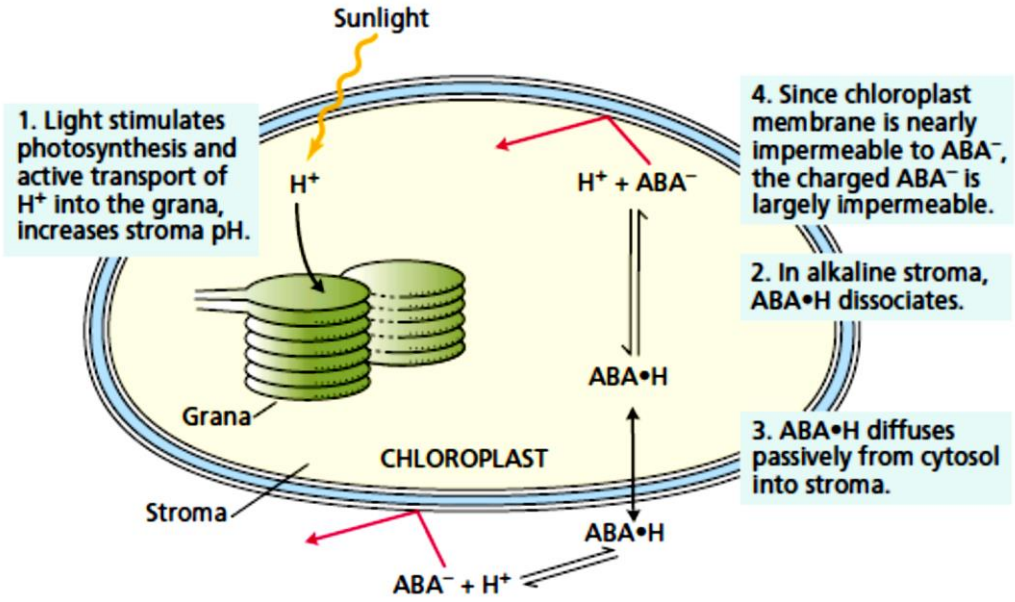


Figure 2.1 Mechanism of ABA in stomatal clsoing

v) Water deficit limits Photosynthesis and carbon dioxide assimilation

Water deficit stress exerts a profound impact on photosynthetic activity by disrupting multiple physiological and biochemical processes (Figure 2.2). Reduced water availability lowers tissue water potential, which triggers abscisic acid (ABA)-mediated stomatal closure, thereby restricting CO_2 influx into the leaf mesophyll. The reduced CO_2 availability limits Rubisco activity and carboxylation efficiency, further aggravated by the accumulation of Rubisco binding inhibitors and diminished activities of key photosynthetic enzymes such as PEP carboxylase, NADP-ME (NADP-dependent Malic Enzyme), FBPase (Fructose-1,6-

bisphosphatase) and PPDK (Pyruvate, Phosphate Dikinase). Concurrently, drought stress induces the excessive generation of reactive oxygen species (ROS), which attack cellular membranes and photosynthetic proteins, leading to impaired photosystem integrity. This imbalance between light absorption and utilization down-regulates non-cyclic electron transport, resulting in reduced NADPH generation and obstructed ATP synthesis. Collectively, these effects culminate in restricted carbon assimilation, metabolic inefficiency, and a marked decline in photosynthetic performance under drought conditions.

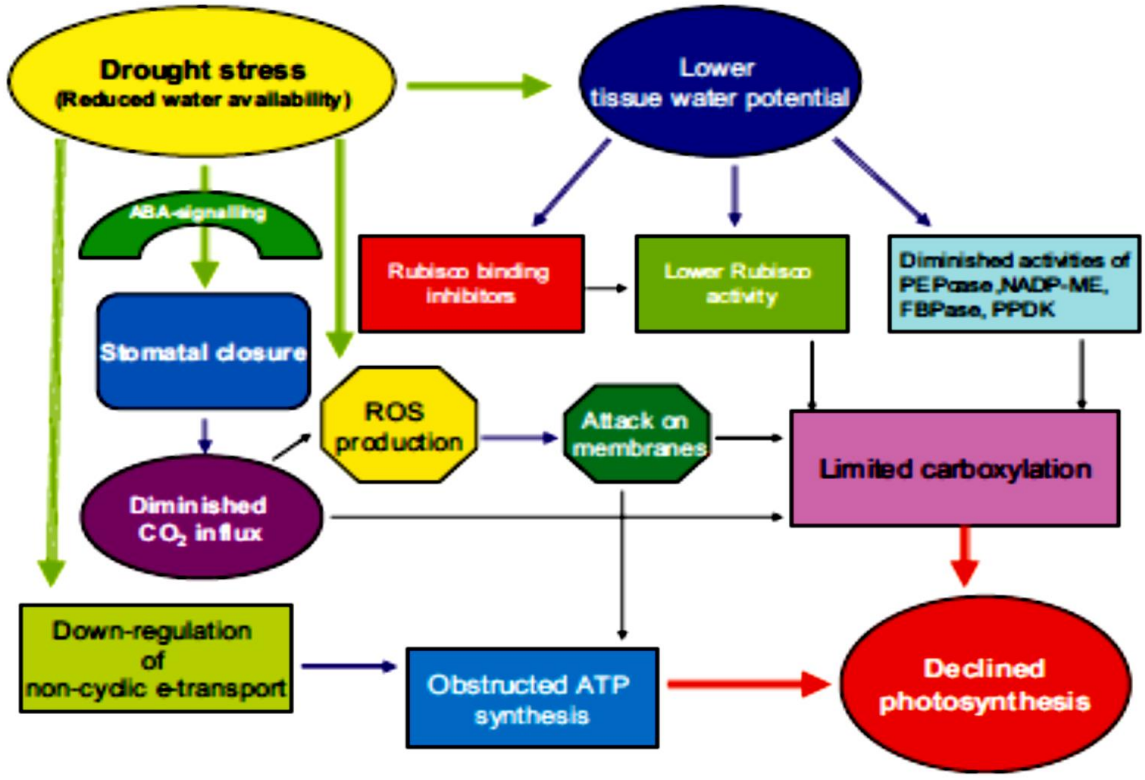


Figure 2.2 Schematic representation of reduced photosynthetic rate under water deficit stress

vi) Water deficit increases resistance to Liquid-Phase water flow

When soil dries, its resistance to the flow of water increases very sharply, particularly near the permanent wilting point. At the permanent wilting point (usually about –1.5 MPa), plants cannot regain turgor pressure even if all transpiration stops. Because of the very large soil resistance to water flow, water delivery to the roots at the permanent wilting point is too slow to allow the overnight rehydration of plants that have wilted during the day. Rehydration is further hindered by the resistance within the plant, which has been found to be larger than the resistance within the soil over a wide range of water deficits. Several factors may contribute to the increased plant resistance to water flow during drying. As plant cells lose water, they shrink. When roots shrink, the root surface can move away from the soil particles that hold the water, and the delicate root hairs may be damaged. In addition, as root extension slows during soil drying, the outer layer of the root cortex (the hypodermis) often becomes more extensively covered with suberin, a water-impermeable lipid (Figure 2.3).

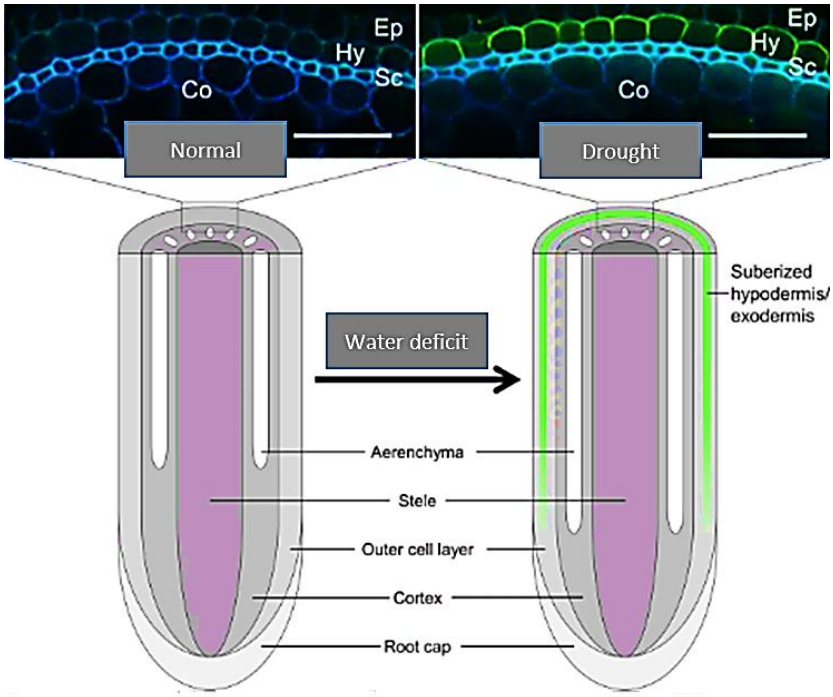


Figure 2.3 Increased suberin under water deficit stress around root cortex

vii) Water Deficit Alters Energy Dissipation from Leaves

Evaporative heat loss lowers leaf temperature. When water stress limits transpiration, the leaf heats up unless another process offsets the lack of cooling. When transpiration decreases and leaf temperature becomes warmer than the air temperature, some of the extra energy in the leaf is dissipated as sensible heat loss. Many arid-zone plants have very small leaves, which minimize the resistance of the boundary layer to the transfer of heat from the leaf to the air. Because of their low boundary layer resistance, small leaves tend to remain close to air temperature even when transpiration is greatly slowed. In contrast, large leaves have higher boundary layer resistance and dissipate less thermal energy (per unit leaf area) by direct transfer of heat to the air. In larger leaves, leaf movement can provide additional protection against heating during water stress. Other factors that can alter the interception of radiation include wilting, which changes the angle of the leaf, and leaf rolling in grasses, which minimizes the profile of tissue exposed to the sun. Absorption of energy can also be decreased by hairs on the leaf surface or by layers of reflective wax outside the cuticle. Leaves of some plants have a gray-white appearance because densely packed hairs reflect a large amount of light. This hairiness, or **pubescence**, keeps leaves cooler by reflecting radiation, but it also reflects the visible wavelengths that are active in photosynthesis and thus it decreases carbon assimilation.

viii) Osmotic stress and crassulacean acid metabolism

Crassulacean acid metabolism (CAM) is very prevalent in succulent plants such as cacti. Some succulent species display facultative CAM, switching to CAM when subjected to water deficits or saline conditions. This switch in metabolism is a remarkable adaptation to stress, involving accumulation of the enzymes phosphoenolpyruvate (PEP) carboxylase, pyruvate–orthophosphate dikinase, and NADP malic enzyme, among others. The CAM metabolism involves many structural, physiological, and biochemical features, including

changes in carboxylation and decarboxylation patterns, transport of large quantities of malate into and out of the vacuoles, and reversal of the periodicity of stomatal movements.

xi) Reduction of growth rate

The first and foremost effect of drought is impaired germination and poor stand establishment. Drought stress has been reported to severely reduce germination and seedling stand. Growth is accomplished through cell division, cell enlargement and differentiation, and involves genetic, physiological, ecological and morphological events and their complex interactions. The quality and quantity of plant growth depend on these events, which are affected by water deficit. Cell growth is one of the most drought-sensitive physiological processes due to the reduction in turgor pressure. Under severe water deficiency, cell elongation of higher plants can be inhibited by interruption of water flow from the xylem to the surrounding elongating cells. Plants respond to water stress in the form of hydraulic signaling, that is, decreased root growth water uptake, water potential, turgidity, and leaf enlargement. As a result of water stress, the water potential and transpiration rate of the plant is decreased. This results in a reduction in cell turgor and relative water content, which damages the plant (Figure 2.4). Drought conditions severely affect plant height, leaf area index, and yield per hectare. Under water stress conditions, the growth of cells is inhibited and root growth is favored over leaf growth. With the increase in water stress in roots, osmotic adjustment occurs rapidly, which allows partial turgor recovery and re-establishment of osmotic gradient for water uptake. Moreover, the loosening ability of the cell wall also decreases. This allows the root to resume its growth under stress conditions. Continuous water stress reduces the carbon exchange rate in plants. This results in a 39% lower yield and 23–33% smaller seeds. Senescence induced by water stress cannot be stopped by relieving the plant from stress. Short periods of stress during seed filling have large effects.

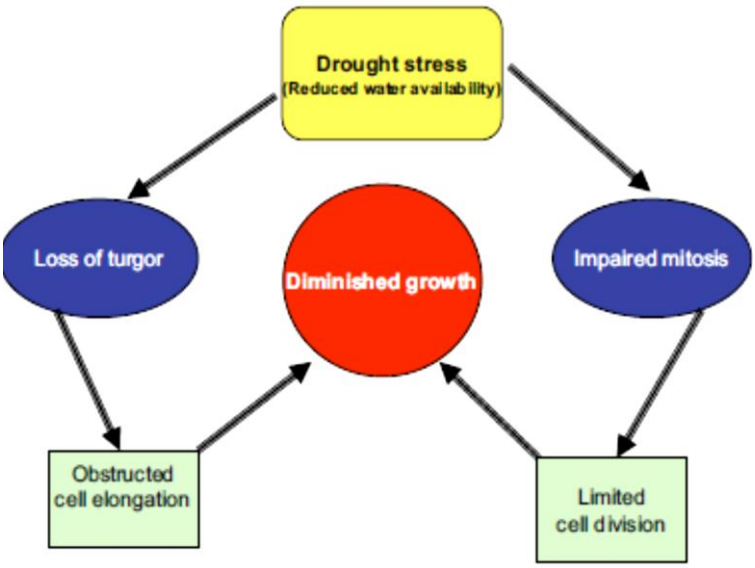


Figure 2.4 Reduction in cell elongation and cell growth under water deficit stress

x) Water relations

Relative water content, leaf water potential, stomatal resistance, rate of transpiration, leaf temperature and canopy temperature are important characteristics that influence plant water relations. The ratio between dry matter produced and water consumed is termed as water-use efficiency. Stomatal opening and closing is more strongly affected. Drought-tolerant species maintain water-use efficiency by reducing the water loss.

xi) Nutrient relations

Decreasing water availability under drought generally results in limited total nutrient uptake and their diminished tissue concentrations in crop plants. An important effect of water deficit is on the acquisition of nutrients by the root and their transport to shoots. Lowered absorption of the inorganic nutrients can result from interference in nutrient uptake and the unloading mechanism, and reduced transpirational flow. In general, moisture stress induces an increase in N, a definitive decline in P and no definitive effects on K.

xii) Stomatal oscillations

The first response of virtually all plants to acute water deficit is the closure of their stomata to prevent the transpirational water loss. This may result in response to either a decrease in leaf turgor and/or water. Drought mainly limits photosynthesis through stomatal closure or metabolic impairment has continued for a long time. This decreases the inflow of CO_2 into the leaves and spares more electrons for the formation of active oxygen species. As the rate of transpiration decreases, the amount of heat that can be dissipated increases. Stomata close progressively as drought progresses, followed by a parallel decline in net photosynthesis.

xiii) Adenosine triphosphate synthesis

Impaired photophosphorylation and adenosine triphosphate synthesis are the main factors limiting photosynthesis even under mild drought. Under drought stress, production of limited nicotinamide adenine dinucleotide phosphate maintains the continuation of electron transport, although the status of the reductant may be high even when the fluxes are small, leading to a more increased demand than supply. Under drought stress, non-cyclic electron transport is down-regulated to match the requirements of decreased nicotinamide adenine dinucleotide phosphate production and cyclic electron transport is activated. This generates a proton gradient that induces the protective process of high-energy-state quenching.

xiv) Assimilate partitioning

Drought stress frequently enhances allocation of dry matter to the roots, which can enhance water uptake. Drought stress decreases the photosynthetic rate, and disrupts the carbohydrate metabolism and level of sucrose in leaves that spills over to a decreased export rate.

xv) Respiration

Severe drought reduced the shoot and root biomass, photosynthesis and root respiration rate. Water deficit in the rhizosphere leads to an increased rate of root respiration, leading to an imbalance in the utilization of carbon resources, reduced production of adenosine triphosphate and enhanced generation of reactive oxygen species. Limited root respiration and root biomass under severe soil drying can improve growth and physiological activity of drought-tolerant over a drought-sensitive cultivar in arid regions.

xvi) Oxidative damage

Exposure of plants to drought stress quite often leads to the generation of reactive oxygen species, including superoxide anion radicals (O^{-2}), hydroxyl radicals (OH), hydrogen peroxide (H_2O_2), alkoxy radicals (RO) and singlet oxygen (O_{12}). Reactive oxygen species may react with proteins, lipids and deoxyribonucleic acid, causing oxidative damage and impairing the normal functions of cells. Many cell compartments produce reactive oxygen species; of these, chloroplasts are a potentially important source because excited pigments in thylakoid membranes may interact with O_2 to form strong oxidants such as O^{-2} or O_{12}. The interaction of O_2 with reduced components of the electron transport chain in mitochondria can lead to reactive oxygen species formation, and peroxisomes produce H_2O_2 when glycolate is oxidized into glyoxylic acid during photorespiration.

15.4 DROUGHT RESISTANCE MECHANISMS

Plants respond and adapt to and survive under drought stress by the induction of various morphological, biochemical and physiological responses. Drought tolerance is defined as the ability to grow, flower and display economic yield under suboptimal water supply. To cope with the drought, tolerant plants initiate defense mechanisms against water deficit.

15.4.1 Morphological mechanisms

Plant drought tolerance involves changes at whole-plant, tissue, physiological and molecular levels. Manifestation of a single or a combination of inherent changes determines the ability of the plant to sustain itself under limited moisture supply.

Escape: Escape from drought is attained through a shortened life cycle or growing season, allowing plants to reproduce before the environment becomes dry. Flowering time is an important trait related to drought adaptation, where a short life cycle can lead to drought escape.

Avoidance: Drought avoidance consists of mechanisms that reduce water loss from plants, due to stomatal control of transpiration, and also maintain water uptake through an extensive and prolific root system. A deep and thick root system is helpful for extracting water from considerable depths. Glaucousness or waxy bloom on leaves helps with maintenance of high tissue water potential, and is therefore considered as a desirable trait for drought tolerance.

Phenotypic flexibility: At a morphological level, the shoot and root are the most affected and both are the key components of plant adaptation to drought. Plants generally limit the number and area of leaves in response to drought stress just to cut down the water budget at the cost of yield loss. Leaf pubescence is a xeromorphic trait that helps protect the leaves from excessive heat load. Hairy leaves have reduced leaf temperatures and transpiration. The possession of a deep and thick root system allowed access to water deep in the soil.

Wax deposition: A common developmental response to water stress is the production of a thicker cuticle that reduces water loss from the epidermis (cuticular transpiration). Although waxes are deposited in response to water deficit both on the surface and within the cuticle inner layer, the inner layer may be more important in controlling the rate of water loss in ways that are more complex than by just increasing the amount of wax present. A thicker

cuticle also decreases CO_2 permeability, but leaf photosynthesis remains unaffected because the epidermal cells underneath the cuticle are non-photosynthetic. Cuticular transpiration, however, accounts for only 5 to 10% of the total leaf transpiration, so it becomes significant only if stress is extremely severe or if the cuticle has been damaged by wind-driven sand.

15.4.2 Physiological mechanisms

Cell and tissue water conservation: Improved tissue water status may be achieved through osmotic adjustment and/or changes in cell wall elasticity. Osmotic adjustment helps to maintain the cell water balance with the active accumulation of solutes in the cytoplasm, thereby minimizing the harmful effects of drought. The osmotic adjustment also facilitates a better translocation of pre-anthesis carbohydrate partitioning during grain filling.

Osmotic Adjustment: Osmotic adjustment, or accumulation of solutes by cells, is a process by which water potential can be decreased without an accompanying decrease in turgor or decrease in cell volume. The change in cell water potential results simply from changes in solute potential (Ψ_s), the osmotic component of Ψ_w. Osmotic adjustment is a net increase in solute content per cell that is independent of the volume changes that result from loss of water. Most of the adjustment can usually be accounted for by increases in concentration of a variety of common solutes, including sugars, organic acids, amino acids, and inorganic ions (especially K^+). Cytosolic enzymes of plant cells can be severely inhibited by high concentrations of ions. The accumulation of ions during osmotic adjustment appears to be restricted to the vacuoles, where the ions are kept out of contact with enzymes in the cytosol or subcellular organelles. Synthesis of compatible solutes like amino acid proline, sugar alcohols (e.g., sorbitol and mannitol), and a quaternary amine called glycine betaine helps plants adjust to increased water deficit stress in the rooting zone. Osmotic adjustment develops slowly in response to tissue dehydration.

Antioxidant defence: Exposure to drought stress leads to the generation of reactive oxygen species (ROS). The ROS may react with proteins, lipids and DNA, causing oxidative damage and impairing the normal functions of cells. The antioxidant defence system in the plant cell includes both enzymatic and non-enzymatic constituents. Amongst the enzymatic components are superoxide dismutase, catalase, peroxidase, and ascorbate peroxidase and glutathione reductase. Upon exposure to abiotic stresses, tolerant cells activate their enzymatic antioxidant system, which then starts quenching the ROS and protecting the cell (Figure 2.5).

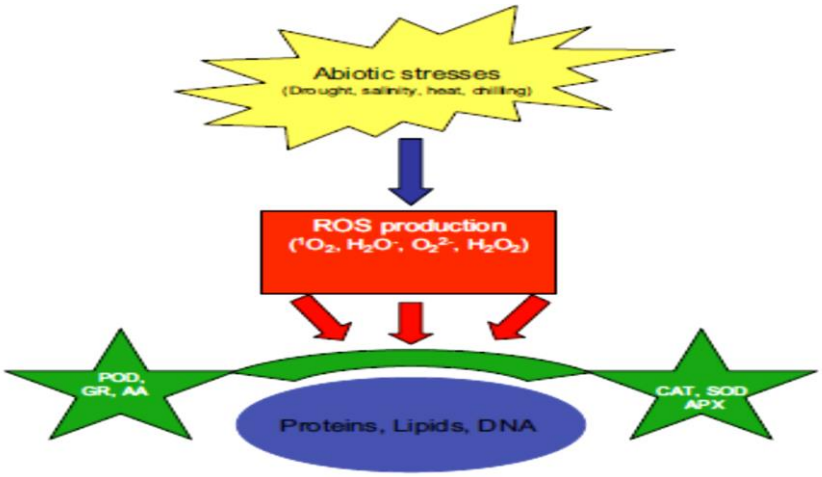


Figure 2.5 Antioxidant defence mechanism of plants against water deficit stress

Cell membrane stability: Biological membranes are the first target of many abiotic stresses. A decrease in cellular volume causes crowding and increases the viscosity of cytoplasmic components. This increases the chances of molecular interactions that can cause protein denaturation and membrane fusion. Proline, glutamate, glycinebetaine, carnitine, mannitol, sorbitol, fructans, polyols, trehalose, sucrose and oligosaccharides stop the membrane degradation.

Plant growth regulators: Plant growth regulators (PGRs) play a pivotal role in enabling plants to withstand water deficit stress by modulating physiological and biochemical processes (Figure 2.6). Among them, abscisic acid (ABA) is the key drought hormone, inducing stomatal closure to reduce water loss, promoting root growth, and activating stress-responsive genes and osmolyte accumulation. In contrast, cytokinins and gibberellins generally decline during drought, suppressing growth and transpiration, thereby conserving energy and water. Auxins enhance root system development to improve water uptake, while ethylene in moderate amounts aids in stress signaling but in excess accelerates senescence. Brassinosteroids, salicylic acid, and jasmonic acid strengthen antioxidant defenses, stabilize membranes, and regulate stress proteins, helping plants maintain cellular integrity. Together, these PGRs coordinate adaptive responses that optimize water use efficiency, balance growth with survival, and improve overall drought tolerance.

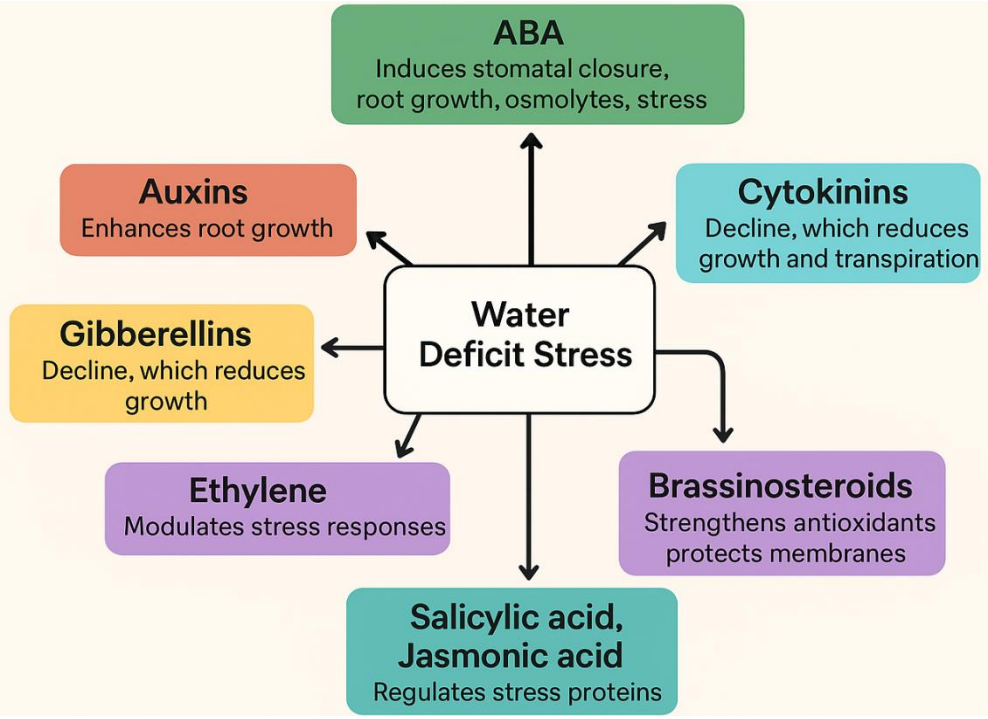


Figure 2.6 Role of plant growth regulators against water deficit stress

15.4.3 Molecular mechanisms

Plant cellular water deficit may occur under conditions of reduced soil water content. Under these conditions, changes in gene expression (up- and down-regulation) take place. Various genes are induced in response to drought at the transcriptional level, and these gene products are thought to function in tolerance to drought.

Osmotic Stress Changes Gene Expression: Accumulation of compatible solutes in response to osmotic stress requires the activation of the metabolic pathways that biosynthesize these

solutes. Several genes coding for enzymes associated with osmotic adjustment are turned on (up-regulated) by osmotic stress and/or salinity, and cold stress. These genes encode enzymes such as Δ′1-Pyrroline-5-carboxylate synthase, a key enzyme in the proline biosynthetic pathway, Betaine aldehyde dehydrogenase, an enzyme involved in glycine betaine accumulation, *myo*-Inositol 6-*O*-methyltransferase, a rate-limiting enzyme in the accumulation of the cyclic sugar alcohol called pinitol. Several other genes that encode well-known enzyme are induced by osmotic stress. The expression of glyceraldehyde-3-phosphate dehydrogenase also increases during osmotic stress. Enzymes involved in lignin biosynthesis are also controlled by osmotic stress. Reduction in the. Other genes regulated by osmotic stress encode proteins associated with membrane transport, including ATPases and the water channel proteins, *aquaporins*. Several protease genes are also induced by stress, and these enzymes may degrade other proteins that are denatured by stress episodes. The protein *ubiquitin* tags proteins that are targeted for proteolytic degradation. Synthesis of the mRNA for ubiquitin increases in *Arabidopsis* upon desiccation stress. In addition, some *heat shock proteins* are osmotically induced and may protect or renature proteins inactivated by desiccation. Genes coding for enzymes such as adenosylmethionine synthase and peroxidases, which may be involved in lignin biosynthesis, have been shown to be controlled by stress. A large group of genes that are regulated by osmotic stress was genes code for **LEA proteins** (late embryogenesis abundant), and they are suspected to play a role in cellular membrane protection. The proteins encoded by these genes are typically hydrophilic and strongly bind water. Their protective role might be associated with an ability to retain water and to prevent crystallization of important cellular proteins and other molecules during desiccation. They might also contribute to membrane stabilization.

Aquaporins: Aquaporins have the ability to facilitate and regulate passive exchange of water across membranes. They belong to a highly conserved family of major intrinsic membrane proteins. In plants, aquaporins are present abundantly in the plasmamembrane and in the vacuolarmembrane. Aquaporins can regulate the hydraulic conductivity of membranes and potentiate a ten- to twenty-fold increase in water permeability. Aquaporins play a major role in overall root water uptake and play a role in cellular osmoregulation of highly compartmented root cells. The over expression of the plasma membrane aquaporin progressively improved the plant vigour under favourable growth conditions. Phosphorylation, calcium and pH are important factors modulating aquaporin activity.

Stress proteins: Synthesis of stress proteins is a ubiquitous response to cope with prevailing stressful conditions including water. Most of the stress proteins are soluble in water and therefore contribute towards the stress tolerance phenomena by hydration of cellular structures. The dehydration-responsive element-binding genes are involved in the abiotic stress signalling pathway. It was possible to engineer stress tolerance in transgenic plants by manipulating the expression of dehydration-responsive element binding genes.

Heat shock proteins: Belong to a larger group of molecules called chaperones. They have a role in stabilizing other proteins' structure. They are reported to serve as molecular chaperones prevent protein denaturation during stress.

LEA proteins: Membrane-stabilizing proteins and late embryogenic abundant proteins. These increase the water binding capacity by creating a protective environment for other proteins or structures, referred to as dehydrins. They also play a major role in the sequestration of ions that are concentrated during cellular dehydration. These proteins help to protect the partner protein from degradation.

Dehydrins: Also known as a group of late embryogenesis abundant proteins, accumulate in response to both dehydration and low temperature.

Signaling and drought stress tolerance: General responses to stress involve signaling stress detection via the redox system, checkpoints arresting the cell cycle and deoxyribonucleic acid repair processes stimulated in response to deoxyribonucleic acid damage.

ABA dependent and ABA independent Regulation of Stress-Responsive Genes

Gene transcription is controlled through the interaction of regulatory proteins (transcription factors) with specific regulatory sequences in the promoters of the genes they regulate. Different genes that are induced by the same signal like desiccation or salinity are controlled by a signaling pathway leading to the activation of these specific transcription factors. Studies of the promoters of several stress-induced genes have led to the identification of specific regulatory sequences for genes involved in different stresses Ex: *RD29* gene contains DNA sequences that can be activated by osmotic stress, by cold, and by ABA. The promoters of ABA-regulated genes contain a six nucleotide sequence element referred to as the **ABA response element** (**ABRE**), which probably binds transcriptional factors involved in ABA-regulated gene activation. The promoters of these genes, which are regulated by osmotic stress in an ABA-dependent manner, contain an alternative nine-nucleotide regulatory sequence element, the **dehydration response element** (**DRE**) which is recognized by an alternative set of proteins regulating transcription. Thus the genes that are regulated by osmotic stresses appear to be regulated either by signal transduction pathways mediated by the action of ABA (**ABA-dependent genes**), or by an **ABA-independent**, osmotic stress–responsive signal transduction pathway (Figure 2.7). At least two signalling pathways have been implicated in the regulation of gene expression in an ABA-independent manner. Transacting transcription factors DREB1 and DREB2 that bind to the DRE elements in the promoters of osmotic stress responsive genes are apparently activated by an ABA independent signalling cascade. Other ABA-independent, osmotic stress responsive genes appear to be directly controlled by the so-called MAP kinase signalling cascade of protein kinases. Other changes in gene expression appear to be mediated via other mechanisms not involving DREBs. This complexity and "cross-talk" found in signalling cascades, exemplified here by both ABA-dependent and ABA independent pathways, is typical of eukaryotic signalling. Such complexity reflects the wealth of interaction between gene expression and the physiological processes mediating adaptation to osmotic stress.

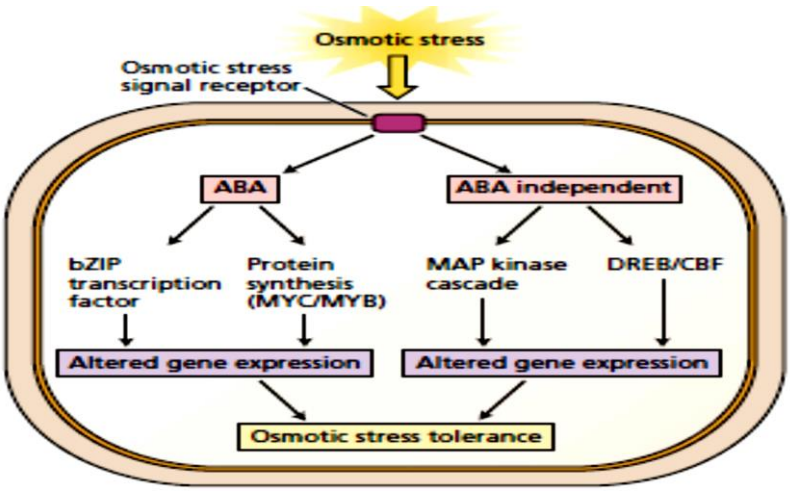


Figure 2.7 ABA mediated stress regulation mechanism

15.5 SUMMARY

Water deficit stress, commonly known as drought stress, is one of the most severe abiotic stresses that limits plant growth and crop productivity. Water deficit stress in plants occurs when water availability is lower than the plant's requirements, leading to physiological, biochemical, and molecular alterations. The immediate response is stomatal closure to reduce transpiration, which simultaneously limits CO_2 uptake and photosynthesis. Prolonged stress results in reduced cell expansion, leaf area, and biomass accumulation, while inducing osmotic adjustment through accumulation of solutes like proline, sugars, and glycine betaine. It also triggers oxidative stress due to excessive reactive oxygen species (ROS), causing membrane damage, lipid peroxidation, and enzyme inactivation. To cope with this, plants adopt several responses such as accumulation of osmolytes (proline, sugars), production of stress proteins, activation of antioxidant enzymes, hormonal regulation (mainly abscisic acid-mediated stomatal closure), and changes in root architecture to improve water uptake. Plants adapt through drought-avoidance mechanisms (deep root systems, leaf rolling, cuticular thickening) and drought-tolerance strategies (antioxidant defense, osmolyte synthesis, and stress-related gene expression). Overall, water deficit stress disrupts plant growth and yield but also activates protective mechanisms to enhance survival under limited water conditions.

15.7 SELF-ASSESSMENT QUESTIONS

1. Define water deficit (drought) stress in plants.
2. What is the role of abscisic acid (ABA) during drought stress?
3. Write a short note on osmotic adjustment under drought conditions.
4. Differentiate between drought escape and drought tolerance strategies.
5. What are LEA (Late Embryogenesis Abundant) proteins and their role in drought tolerance?
6. Explain the physiological, biochemical, and molecular effects of water deficit stress on plants.
7. Write an essay on osmotic adjustment and its importance in plant drought tolerance.
8. Describe the antioxidant defense mechanisms activated during water deficit stress.

15.8 REFERENCE BOOKS

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Dr K.Babu

LESSON- 16

STRUCTURAL, PHYSIOLOGICAL, BIOCHEMICAL AND MOLECULAR RESPONSES OF PLANTS TO SALINITY STRESS

OBJECTIVE:

Students are able to know the physiological, biochemical, and molecular impacts of salinity on plants and to highlight the adaptive mechanisms plants employ to survive and sustain growth under salt stress conditions.

STRUCTURE OF THE LESSON:

16.1 INTRODUCTION

Soil salinity, mainly caused by high concentrations of soluble salts such as sodium chloride, is a major agricultural problem, especially in arid and semi-arid regions where it affects about 25% of farmland. It reduces plant growth and yield by lowering water uptake, inducing osmotic and ion toxicity stress, generating reactive oxygen species (ROS), and

disrupting nutrient balance. Excess Na^+ and Cl^- ions impair enzyme activity, damage cellular structures, and accelerate leaf senescence. Plants respond in two phases: an early ion-independent phase marked by reduced water relations and stomatal closure, and a later ion-dependent phase involving toxic ion accumulation in leaves. To cope, plants adopt mechanisms such as ion exclusion, tissue tolerance, osmotic adjustment, antioxidant production, and secondary metabolite synthesis, which help maintain turgor, photosynthesis, stomatal function, and overall water status, allowing better growth under saline conditions.

16.2 EFFECT OF SALINITY ON PLANTS

Salinity composes stress by damaging ionic and osmotic balances in plants. Osmotic stress caused by increasing the amount of salt in soil, decreases the amount of water that plant use and as a result physiological drought occurs. After these conditions, ionic stress occurs in the plant with deterioration of plant ion balance. Na and Cl ions which increases in medium with ionic stress, get in competition with essential nutrients such as K^+, Ca^{2+}, Mg^{2+} lead to nutrient deficiency in plant. While the direct effect of salinity is osmotic and ionic stresses, deteriorations in structure and synthesis of toxic components composes secondary effect (Figure 2.8).

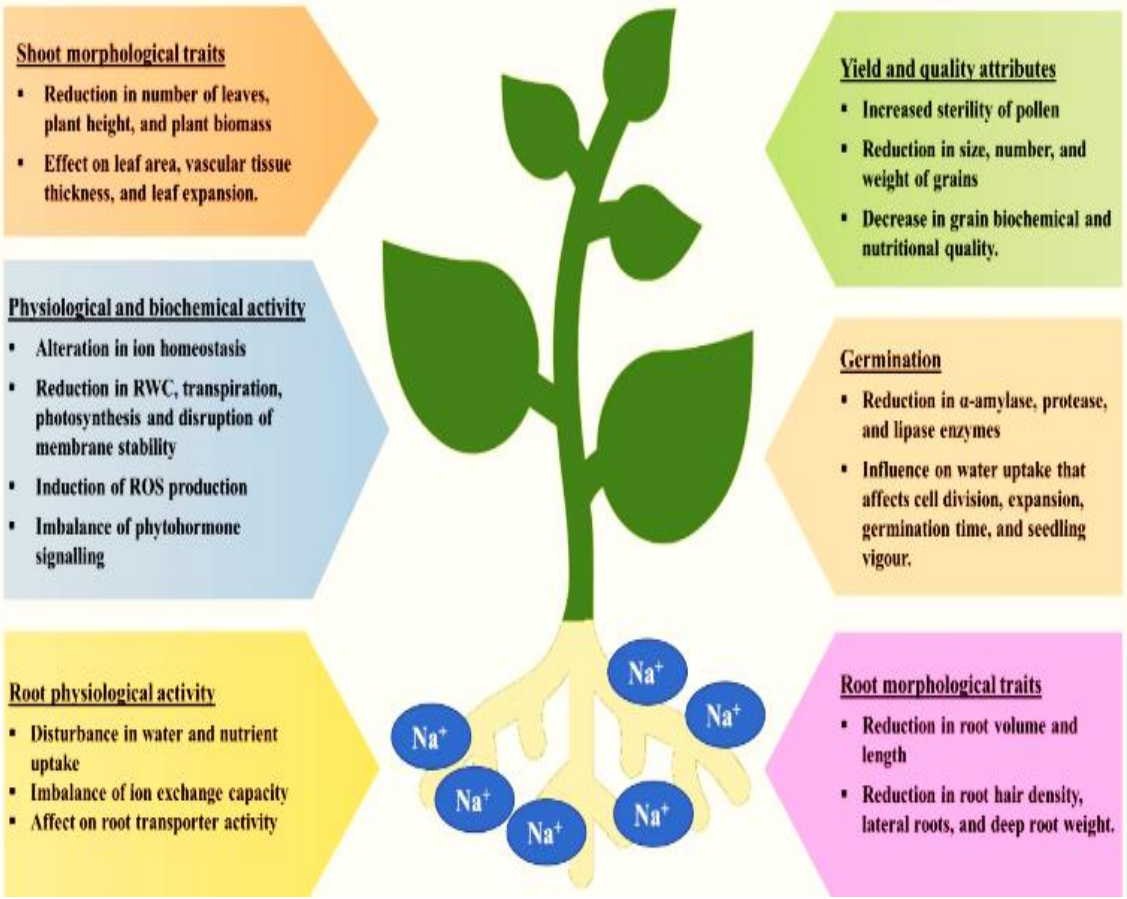

Figure 2.8 Detrimental effects of salinity stress on plant parts at different growth stages

16.2.1 Morphological effects

The main secondary factors caused by NaCl are, complication in taking of K+ into cells (it is efficient in closure of stomatas), decreasing photosynthetic activity, generation of

reactive oxygen species (ROS) and programmed cell deaths. K element is one of the most vital elements for plants for this reason Na+ ions compete with K+ ions for getting into the cell. Na+ composes stress with blocking K+ influx into the cell. Ion and hyperosmotic stress causes secondary metabolic effects in plant and the plant has to decrease these stresses for maintain its development.

Effect on germination and seedling stage

Seed germination is a multi-stage developmental process that is influenced by both internal and external factors. Impact on seed germination under salinity stress may be attributed to the delayed absorption of water and a decline in the activity of α-amylase, an enzyme involved in starch hydrolysis. Salinity lowers the soil osmotic potential relative to the internal osmotic potential of seed, which inhibits the absorption of water during seed imbibition (Figure 2.8). As a consequence, the seed germination rate is reduced and the seed germination period is delayed. Even after germination, salinity may also have detrimental effect on embryo viability due to the excess accumulation of Na^+ and Cl^- ions. Furthermore, salt stress increases the generation of reactive oxygen species (ROS) and oxidative damages, which disrupt different macromolecules. Therefore, a decrease in α-amylase activity results in a significant reduction in the transfer of sugars, which restricts the embryo's growth and development.

Growth and development

Like many other abiotic stresses, salt stress suppresses plant growth, and the rate of growth reduction depends on several factors, such as plant species, developmental stage, and the concentration of salt. Stunted growth is an adaptive mechanism for survival, which allows plants to combat salt stress. Salt stress might reduce the expression of key regulatory genes involved in cell cycle progression (e.g., cyclin and cyclin-dependent kinase), leading to decreased cell numbers in the meristem and a growth inhibition which impacts the plant's ability to absorb nutrients and water efficiently. After the occurrence of salinity stress, the lateral shoot enlargement is affected, leading to apparent differences in overall growth and injury between salt-stressed plants and their nonstressed plants. This response is due to changes in the cell–water relation resulting from osmotic changes outside the root (osmotic effect). The osmotic effect leads to a reduction in the capability of plants to absorb water.

Root and shoot growth

Root length, root length density and thick roots which are the features of the root, are very vital in development of subsoil parts of plant by taking the existing water. Roots are one of the vulnerable parts of the plant. Under salt stress reduction in root length and shoot growth is observed. Also, high concentration of salinity causes reduction in leaf fresh and dry weights, with low humidity it causes reduction in shoot and root growth on plants. With affecting stomata, salt stress stops permanence of stomatal reactions. Formation of root nodules, plant sprouts and leaves are affected from salt stress.

16.2.2 Cell level effect

Organelle level effect

The most affected organelle in plant from salt stress is chloroplast. Stress mostly affects thylakoids and stroma in chloroplast. Chloroplasts tend to generate reactive oxygen species such as H_2O_2, O^{2-}, OH^-. Reactive oxygen species seriously affects plant metabolic

activities by causing oxidative damage to lipids that result in protein breakdown and membrane lipid peroxidation, causing the thylakoids to swell and turn into a wavy shape. The stress caused by salt, also results in starch accumulation in chloroplast. Another negative situation caused by salt stress is deterioration of grana lamellae. Salt stress disrupts electrical charges that composes grana lamellae by changing ionic composition. As a result of stress mitochondria, the another organelle affected from salt stress, is exposed to negative effects such as structural fragmentations, accelerations in vacuole forming, swelling and decrease in crystal.

Effect of ion toxicity

Accumulation of Na+ and Cl− ions in plant makes negative effect by developing competitive system with limiting intake of the other ions into the plant. Soluble salts with high concentrations in soil can cause physiological drought. With accumulation of soluble salts in plant root area, decrease in plant water intake occurs. Accumulated salts in root area creates ion imbalance in cells by getting into plant cells and this imbalance causes growth problems in plant tissues such as leaves, seeds etc. Excessive Na^+ accumulation in plants causes necrosis in old leaves and high concentrations of Na^+ ions in shoots cause metabolic and osmotic problems. Accumulation of soluble salts in high concentrations in soil causes negative effects.

16..2.3 Ionic imbalance and salinity mediated nutritional deficiencies

Some ions can also act as plant nutrients, such as K^+ and SO_4^{2-}, while Na^+ is not considered to be an essential plant nutrient. Thus, soil salinity is often measured in terms of Na^+ and Cl^- . Plants are affected by salinity in three ways. Because of its low osmotic potential, salt makes it difficult for plants to extract water from the soil, subjecting plants to osmotic stress, which limits growth and reduces yield. The Na^+ and Cl^- ions when absorbed and accumulated into the tissues by plants at excess concentrations from soil cause cytotoxicity which eventually result in leaf firing, reduced growth, and finally plant death. Moreover, high levels of Na^+ decrease the availability of other ions such as K^+, Ca^{2+}, and Mg^{2+} due to the cation competition, which can lead to nutrient deficiencies. During salinity, the plant takes up more Na^+ than K^+ as the amount of Na^+ in the growth medium increases, increasing K^+ efflux from the cell and raising the Na/K ratio (Figure 2.9). During salt stress, excessive Na^+ influx encourages ion channel disruption, nutrient replacement and membrane depolarization, leading to abnormalities in nutrient uptake and assimilation. Salinity stress significantly reduces the root surface area by lowering root hair density and root hair length which are directly proportional to nutrient uptake. Essential elements including Ca^{2+}, Mg^{2+}, Fe^{2+}, and Zn^{2+}, which are impacted by salt stress, are required for normal root growth. Therefore, the decrease in root growth can further affect the intake of Ca^{2+}, Mg^{2+}, Fe^{2+}, and Zn^{2+} .

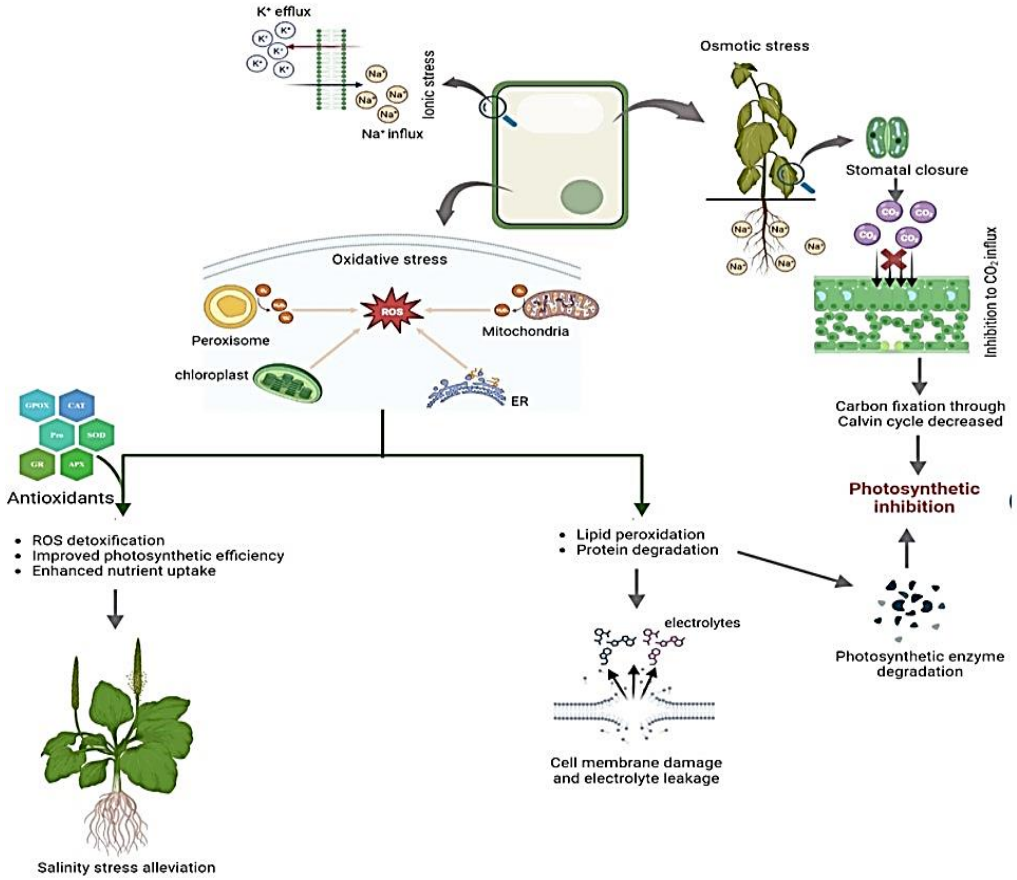

Figure 2.9 Different physiological alterations in plants under salinity and role of antioxidants in stress alleviation

16.2.4 Effect on photosynthesis

Photosynthesis is negatively affected by high and low salt concentrations. Various factors, including impaired chlorophyll biosynthesis, altered enzymatic activity, stomatal closure, reduced CO_2 supply, and damaged photosynthetic apparatus, are correlated with a salt-induced photosynthetic reduction. The decline in chlorophyll content has been reported under salt-stress conditions due to increased oxidation and degradation of chlorophyll initiated by the accumulation of reactive oxygen species (ROS), and the chlorophyll reduction is proportional to the level of salinity. First reason is reduction in cell permeabilization of CO_2 as a result of dehydration of membranes. High concentration of salt in soil creates high osmotic potential in plant by limiting water reaching of plant but with decrease in water potential osmotic stress in plant occurs. Photosynthetic electron transport is affected negatively. Pseudocyclic electron transport resulting from inhibiting the electron transport chain causes an excessive production of ROS. Consequently, ROS alters photosynthetic proteins and the photosystem assembly. In addition, exposure to short-term salt stress at higher concentrations disturbs the dynamics of the chloroplast ultrastructure by inducing thylakoid swelling and starch accumulation.

16.2.5 Closure of stomata

High concentration salinity in soil causes osmotic stress formation by limiting water availability of plant with roots whereas closure of stomatas is a first response of plants. This response of plant limits transpiration and as a result stomata conductivity decreases. Closure

of stomata happens two ways as hydroactive closure and hydropassive closure. Plants synthesis chemical signal molecules in occurring of hydroactive closure. ABA is one of the important synthesized chemical signal molecules and it is effective in plant growth and creating water balance. Under low water potential ABA molecules are transported into stomata by roots and old leaves via xylem. Low water potential is sensed by root tip and ABA molecules are synthesized at the root and transported to shoots with the help of xylem. The reason of synthesis of ABA molecules is ensuring regulation of stomata conductivity in low water potential conditions and as a result of this situation, it causes decrease in leaf water content.

16.3 OXIDATIVE STRESS CAUSED BY SALINITY

Along with its direct effects on plants, salinity frequently results in an excessive build-up of reactive oxygen species (ROS), which can interact with other essential components of plant cells and cause oxidative damage in plants, such as DNA damage, lipid peroxidation, enzyme inactivation, protein oxidation, hormone and nutritional imbalances. ROS are primarily produced in the chloroplasts, mitochondria, endoplasmic reticulum, cytosol, and peroxisome (Figure 2.9). Stomatal closure caused by salt stress can decrease the amount of available carbon dioxide in the leaves and thus, induces photosynthetic inhibition. The light reactions in the chloroplast have a pivotal role in the production of a majority of ROS such as superoxide ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\bullet}$), and singlet oxygen ($^1O_2$). The complex nature of salt stress coupled with water deficit have a variety of detrimental effects on plant metabolic process which in turn produce ROS that affect plant systems. They also reported reduced ascorbate (AsA) to dehydroascorbate (DHA) ratio, along with an increase of methylglyoxal (MG) content, which collectively contribute to oxidative damage. Greater levels of H_2O_2, as well as higher MDA and overproduced MG were also observed in seedlings under salt stress.

16.4 EFFECT ON YIELD AND YIELD COMPONENTS

During the reproductive stage, Na^+ is excluded from leaf blades by the class I high-affinity K^+ transporter (HKT) family, affecting sodium ion homeostasis under salinity stress. It was also found that grain dry matter and the K^+/Na^+ ratio was significantly correlated with grain filling rate and duration under salt stress. Changes in water relations, transpiration, nutritional imbalances, stomatal conductance, and oxidative damage due to salt stress all contribute to a drop in yield. By altering morpho-physiological and biochemical processes, salinity reduces agricultural yield and production.

16.5 RESPONSES AND ADAPTATIONS OF PLANT TO SALT STRESS

In high salinity soils, plants develop a range of morphological, physiological and biochemical adaptations. In addition to ion homeostasis and compartmentalization, the principal responses include biosynthesis of compatible solutes, osmo-protectants, antioxidant compounds, polyamines, and nitrous oxide, as well as regulation of phyto-hormones. The following sections discuss recent advances in elucidating these mechanisms.

16.5.1 Ion uptake, transport, compartmentalization, and homeostasis

Plant organs are unable to function when their tissues or cells contain excessive Na^+ or Cl^- concentrations. Increased Na^+ concentration usually results in a decrease in K^+, and it may be

critical for tolerance to maintain cytosolic K^+ levels at an acceptable level or to maintain 'homeostasis'. The toxic effect of Na^+ may also be a result of its competition with K^+ for enzymes that require K^+, such that the ratio of cytoplasmic Na^+ to K^+ may be more important than the concentration of Na^+ itself. Neither glycophytes nor halophytes can tolerate high salt concentrations in their cytoplasm. However halophytes have developed mechanisms to sequester these ions. Na^+ ion exclusion or compartmentalization's are essential for normal plant growth under salinity stress. During salinity stress, cell membranes and their associated components regulate ion uptake and transport within the cytosol. This results in either excess salt being transported to the vacuole or sequestered in older tissues that eventually senesce, thereby protecting plants from salt stress. Carrier proteins, channel proteins, antiporters, and symporters all take part in ion transport. Some well characterized transporters include Na^+ transporter *AtHKT1:1*, Na^+/H^+ antiporter *AtNHX1*, K^+/Na^+ symporter *TaHKT1* and Na^+/H^+ antiporter *SOS1*. These antiporters facilitate the compartmentalization of excess ions through the movement of Na^+ ions into vacuoles from the cytoplasm. After Na^+ ions enter the cytoplasm in excess, antiporters transport them into vacuoles. Vacuolar membrane H^+ pumps exist in two forms: the vacuolar type H^+-ATPase (V-ATPase) and the vacuolar pyro-phosphatase (V-PPase). V-ATPase being the predominant form which generate motive forces across the vacuolar membrane. The ability of the plant to survive under high salinity depends to a great extent on its V-ATPase activity.

16.5.2 Osmoprotection via compatible solutes

Compatible solutes, also called osmolytes are uncharged, polar, and soluble molecules which usually do not interfere with the cellular metabolism even at high concentrations. Organic osmolytes are synthesized and accumulated in varying amounts amongst different plant species to adjust osmotic potentials and protect cells. They are most commonly proline, glycine betaine, sugars, and polyols. Under salinity stress, the concentrations of cysteine, arginine, and methionine, which represent about 55% of total free amino acids, decreased, whereas proline concentration increased. Additionally, proline accumulated in the intracellular space during salt stress also serves as an organic nitrogen reserve during stress recovery. In salt-stressed plants, sugars like glucose, fructose, fructans, and trehalose are also accumulated. These carbohydrates facilitate stress mitigation via, osmoprotection, and neutralization of reactive oxygen species. Trehalose accumulation is not only a carbohydrate reserve but also a protective mechanism against several stresses including salinity. Compatible solutes stabilize cellular structures and enzymes, act as metabolic signals, and scavenge ROS.

16.5.3 Antioxidant regulation

Abiotic stresses including salinity result in electron overflow, deregulation, and even disruption of electron transport chains (ETCs) in chloroplasts and mitochondria. ROS produced under salinity stress are scavenged by enzymatic oxidants (SOD- Superoxide dismutase, APX- Ascorbate peroxidise, GPX- glutathione reductase, CAT- catalase, PPO- polyphenol oxidase, MDHA- monodehydroascorbate, MDHAR- monodehydroascorbate reductase etc.) as well as non-enzymatic antioxidants (reduced glutathione, flavanoids, phenolics, α-tocopherol, alkaloids etc.) which protect the plants from oxidative damage (Figure 2.9). Among these, Ascorbate peroxidase (APX) and glutathione reductase (GR) are important antioxidant enzymes positively related to salt tolerance. At lower concentration, ROS act as signaling molecules which initiates complex cascade of pathways and interactions. Of these, MAPK (mitogen-activated protein kinase) and salt overly sensitive

(SOS) signaling pathway cascades are important mediators of osmotic, ionic and ROS homoeostasis. ROS signals, through these pathways, trigger antioxidant defense mechanisms and scavenging of ROS.

16.5.4 Role of polyamines

Polyamines (PA) are cationic, aliphatic, and low molecular weight molecules which play a variety of roles in normal growth and development, including cell proliferation, morphogenesis, and growth of flowers and fruits. Plants show higher tolerance to stresses when polyamine levels rise. Under salinity stress, polyamines contribute to cellular responses by modulating ROS homeostasis.

16.5.5 Roles of nitric oxide

The molecule Nitric Oxide (NO) is a small volatile gas that plays an important role in many important plant processes, including regulating root growth, respiration, stomata closure, flowering, cell death, seed germination, and stress responses. Many redox-regulated genes are induced by NO directly or indirectly. By interacting with lipid radicals, NO prevents lipid oxidation, scavenging superoxide radicals and forming peroxynitrite that can be neutralized by other cellular processes. Additionally, it activates antioxidant enzymes (SOD, CAT, GPX, APX, and GR). NO has been demonstrated to mediate salt stress tolerance in plants by counteracting germination inhibition, negating inhibition of growth and by its role in ion-homeostasis.

16.5.6 Hormonal regulation of salinity tolerance

Among the well characterized plant hormones, abscisic acid (ABA), salicylic acid, jasmonic acid, and ethylene are considered as stress response hormones. As a consequence of osmotic stress and water deficit, salt stress increases ABA production in vascular tissues and its distribution in roots and shoots. There is a positive association between ABA accumulation and salinity tolerance. Furthermore, plants can mitigate salinity stress by brassinosteroids. Brassinosteroid can enhance the antioxidant enzymes SOD, POX, APX, and GPX and non-enzymatic antioxidant compounds such as tocopherol, ascorbate, and reduced glutathione.

16.6 SUMMARY

Soil salinity, mainly due to excess sodium chloride, is a major constraint to agriculture, particularly in arid and semi-arid regions, where it affects water uptake, nutrient balance, and overall plant growth. Salinity imposes both osmotic and ionic stresses that reduce seed germination, seedling vigor, root and shoot development, photosynthesis, and yield, while excess Na^+ and Cl^- ions cause ion toxicity, nutrient deficiencies, and premature senescence. At the cellular level, chloroplasts, mitochondria, and membranes are damaged by oxidative stress through overproduction of reactive oxygen species (ROS). Plants respond through morphological, physiological, and biochemical mechanisms, including ion exclusion, compartmentalization, osmotic adjustment via compatible solutes (e.g., proline, sugars, glycine betaine), antioxidant defense systems, and hormonal regulation (ABA, salicylic acid, jasmonic acid, brassinosteroids). Additional protective roles are played by polyamines, nitric oxide, and secondary metabolites, which collectively enhance tolerance. Despite these

adaptations, salinity significantly reduces crop yield and quality, making salt stress management crucial for sustainable agriculture.

16.7 SELF-ASSESSMENT QUESTIONS

1. What are the two main phases of plant response to salinity?
2. Mention two structural/organellar changes in chloroplasts due to salt stress.
3. What role does abscisic acid (ABA) play in salinity tolerance?
4. Explain how Na^+ ions interfere with K^+ uptake in plants.
5. Explain the physiological and biochemical changes in plants under salinity stress.
6. Describe the effect of salinity on seed germination and seedling development.
7. Discuss organelle-level damage in plants under salt stress, with emphasis on chloroplasts and mitochondria.
8. Explain oxidative stress caused by salinity and the role of antioxidants in stress alleviation.
9. Discuss various plant mechanisms of salt tolerance (ion exclusion, osmotic adjustment, antioxidant regulation, hormonal signaling).
10. Write an essay on the role of polyamines, nitric oxide, and phytohormones in mitigating salt stress.
11. Explain how salinity reduces crop yield and the strategies plants adopt to maintain productivity under stress.

16.8 SUGGESTED READINGS

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Dr K.Babu

LESSON -17

STRUCTURAL, PHYSIOLOGICAL, BIOCHEMICAL AND MOLECULAR RESPONSES OF PLANTS TO TEMPERATURE STRESS

OBJECTIVE:

Students are able to know the effect of temperature on plants and plant responses to temperature stress.

STRUCTURE OF THE LESSON:

17.1 INTRODUCTION

High-temperature (HT) stress, defined as exposure to temperatures above a plant's physiological threshold for a duration sufficient to cause irreversible damage. Plant responses to HT stress vary depending on the severity, duration of exposure, and species. Plants generally thrive within a narrow thermal range of –10 °C to +60 °C. Heat stress induces a wide array of negative changes, including the overproduction of reactive oxygen species (ROS), leading to oxidative stress. To cope, plants employ several strategies such as structural modifications, metabolic reprogramming, and signaling mechanisms. They produce compatible solutes to stabilize proteins and membranes, adjust osmotically to maintain cell turgor, and enhance antioxidant defenses to restore redox homeostasis. At the molecular level, heat stress alters gene expression patterns, activating genes encoding osmoprotectants, detoxifying enzymes, transporters, and regulatory proteins. These adjustments contribute to short-term acclimation and, in the long term, adaptation, enabling plants to endure and survive under elevated temperatures.

17.2 PLANT RESPONSES TO HEAT STRESS

Plant responses to HT vary with the degree of temperature, duration and plant type. At extreme HT, cellular damage or cell death may occur within minutes, which may lead to a catastrophic collapse of cellular organization. Heat stress affects all aspects of plant processes like germination, growth, development, reproduction and yield. Heat stress differentially affects the stability of various proteins, membranes, RNA species and cytoskeleton structures, and alters the efficiency of enzymatic reactions in the cell for which the major physiological processes obstacle and creates metabolic imbalance.

17.2.1 Growth

Among the growth stages of plant the germination is affected first of all. Heat stress exerts negative impacts on various crops during seed germination though the ranges of temperatures vary largely on crop species. Reduced germination percentage, plant emergence, abnormal seedlings, poor seedling vigour, reduced radicle and plumule growth of geminated seedlings are major impacts caused by heat stress. At very HT (45 °C) the rate of germination was strictly prohibited and caused cell death and embryos for which seedling establishment rate was also reduced. Plant height, number of tillers and total biomass were reduced in various species in response to HT. High temperature causes loss of cell water content for which the cell size and ultimately the growth is reduced. Reduction in net assimilation rate (NAR) is also another reason for reduced relative growth rate (RGR) under HT. The morphological symptoms of heat stress include scorching and sunburns of leaves and twigs, branches and stems, leaf senescence and abscission, shoot and root growth inhibition, fruit discoloration and damage. Damage to leaf-tip and margins, and rolling and drying of leaves, necrosis, was observed due to HT stress. High temperatures may alter the total phenological duration by reducing the life period. Increases in temperatures 1–2 °C than the optimum result in shorter grain filling periods and negatively affect yield components. At extreme heat stress plants can show programmed cell death in specific cells or tissues may

occur within minutes or even seconds due to denaturation or aggregation of proteins. Moderately HTs plants showed extended period of life and cause gradual death. Both types of injuries or death can lead to the shedding of leaves, abortion of flower and fruit, or even death of the entire plant.

17.2.2 Photosynthesis

Photosynthesis is one of the most heat sensitive physiological processes in plants. High temperature has a greater influence on the photosynthetic capacity of plants especially of C_3 plants than C_4 plants. In chloroplast, carbon metabolism of the stroma and photochemical reactions in thylakoid lamellae are considered as the primary sites of injury at HTs. Thylakoid membrane is highly susceptible to HT. Major alterations occur in chloroplasts like altered structural organization of thylakoids, loss of grana stacking and swelling of grana under heat stress. Again, the photosystem II (PSII) activity is greatly reduced or even stops under HTs. Heat shock reduces the amount of photosynthetic pigments. The ability of plant to sustain leaf gas exchange and CO_2 assimilation rates under heat stress is directly correlated with heat tolerance. Heat markedly affects the leaf water status, leaf stomatal conductance (*gs*) and intercellular CO_2 concentration. Closure of stomata under HT is another reason for impaired photosynthesis that affects the intercellular CO_2. The decline in chl pigment also is a result of lipid peroxidation of chloroplast and thylakoid membranes due to heat stress (40/30 °C, day/night). Photosystem II photochemistry (Fv/Fm ratio) and *gs* were also reduced under the same stress condition. Some other reasons believed to hamper photosynthesis under heat stress are reduction of soluble proteins, Rubisco binding proteins (RBP), large-subunits (LS), and small-subunits (SS) of Rubisco in darkness, and increases of those in light. High temperature also greatly affects starch and sucrose synthesis, by reduced activity of sucrose phosphate synthase, ADP-glucose pyrophosphorylase, and invertase. Heat imposes negative impacts on leaf of plant like reduced leaf water potential, reduced leaf area and pre-mature leaf senescence which have negative impacts on total photosynthesis performance of plant. Under prolonged heat stress depletion of carbohydrate reserves and plant starvation are also observed.

17.2.3 Reproductive Development

During reproduction, a short period of heat stress can cause significant decrease in floral buds and flowers abortion. Even heat spell at reproductive developmental stages plant may produces no flowers or flowers may not produce fruit or seed. The reasons for increasing sterility under abiotic stress conditions including the HT are impaired meiosis in both male and female organs, impaired pollen germination and pollen tube growth, reduced ovule viability, anomaly in stigmatic and style positions, reduced number of pollen grains retained by the stigma, disturbed fertilization processes, obstacle in growth of the endosperm, proembryo and unfertilized embryo. HT treatment (>33 °C) at heading stage significantly reduced anther dehiscence and pollen fertility rate, leading to reduction in the number of pollens on the stigma. High temperature often causes excessive ethylene (Eth) production and leads to male sterility of pollens. The ethylene is hypothesized to inhibit the key enzymes in sugar–starch metabolism which weaken sink strength and restrict grain filling and ultimately produce sterile grain. Higher temperatures affect the grain yield mostly through affecting phenological development processes. High temperature decreased grain length, width, and seed weight in plants. It also increased grain nitrogen (N) concentration which was inversely related to grain weight. All of these factors contributed to reduced yield by 90%.

17.2.4. Oxidative Stress

Different metabolic pathways are depended upon enzymes which are sensitive to various degrees of HTs. Heat stress might uncouple enzymes and metabolic pathways which cause the accumulation of unwanted and harmful ROS most commonly singlet oxygen ($^1O_2$), superoxide radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\cdot}$) which are responsible for oxidative stress. The reaction centers of PSI and PSII in chloroplasts are the major sites of ROS generation though ROS are also generated in other organelles viz. peroxisomes and mitochondria. A linear relationship exists between maximal efficiency of PSII and the accumulated ROS. It is suggested that because of thermal damage to photosystems under such HTs less absorbtion of photon occurs. In such stress conditions, if photon intensity is absorbed by PSI and PSII, the excess of which is required for CO_2 assimilation are considered as surplus electrons, those serve as the source of ROS. Among the ROS, $O_2^{\cdot-}$ is formed by photooxidation reactions (flavoprotein, redox cycling), through Mehler reaction in chloroplasts, during mitochondrial ETCs reactions and glyoxisomal photo respiration, by NADPH oxidase in plasma membranes, xanthine oxidase and membrane polypeptides (Figure 2). Hydroxyl radical is formed due to the reaction of H_2O_2 with $O_2^{\cdot-}$ (Haber- Weiss reaction), reactions of H_2O_2 with Fe^{2+} (Fenton reaction) and decomposition of O_3 in apoplastic space. Singlet oxygen is formed during photoinhibition, and PS II electron transfer reactions in chloroplasts.

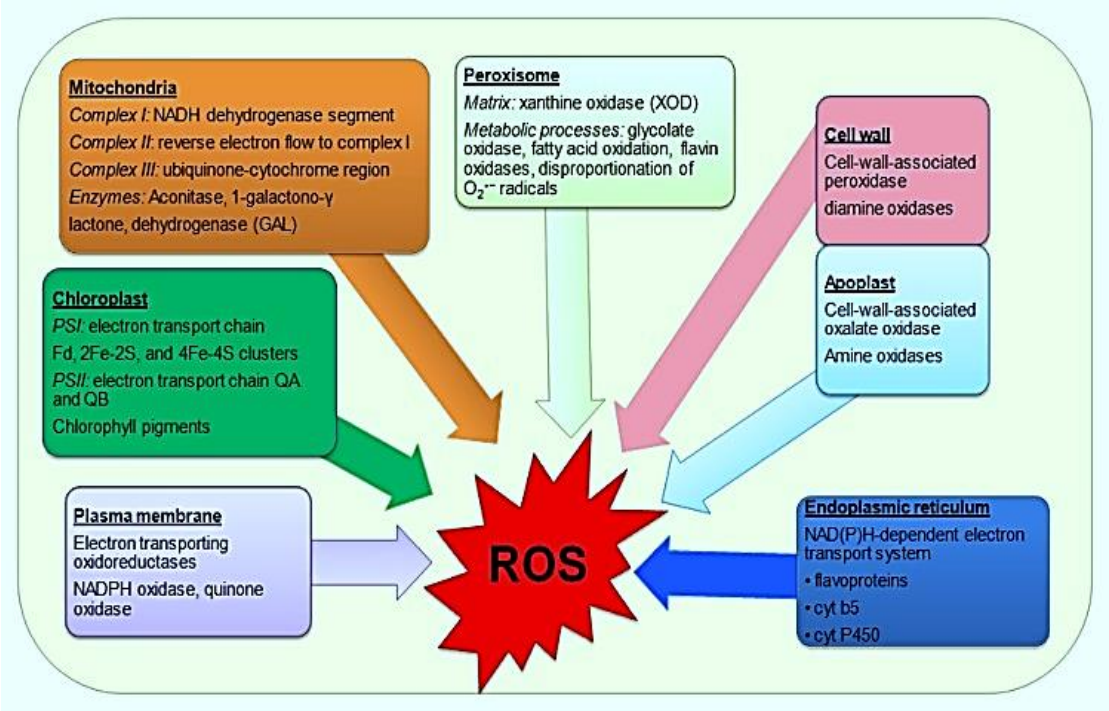

Figure 3.0 Sites of production of reactive oxygen species in plants

17.3 PLANT ADAPTATION TO HEAT STRESS

Living organisms can be classified into three groups, subject to the preferred temperature of growth (Figure 3.1). On the basis of thermotolerance, Larcher classified all the plant species into three groups (Figure 3) (a) Psychrophiles - which grow optimally at low temperature ranges between 0 and 10 °C; (b) Mesophyles - which favor moderate temperature and grow well between 10 and 30 °C; and (c) Thermophyles - which grow well

between 30 and 65 °C or even higher. There is a great variation among the plant species in terms of their response and tolerance to HT.

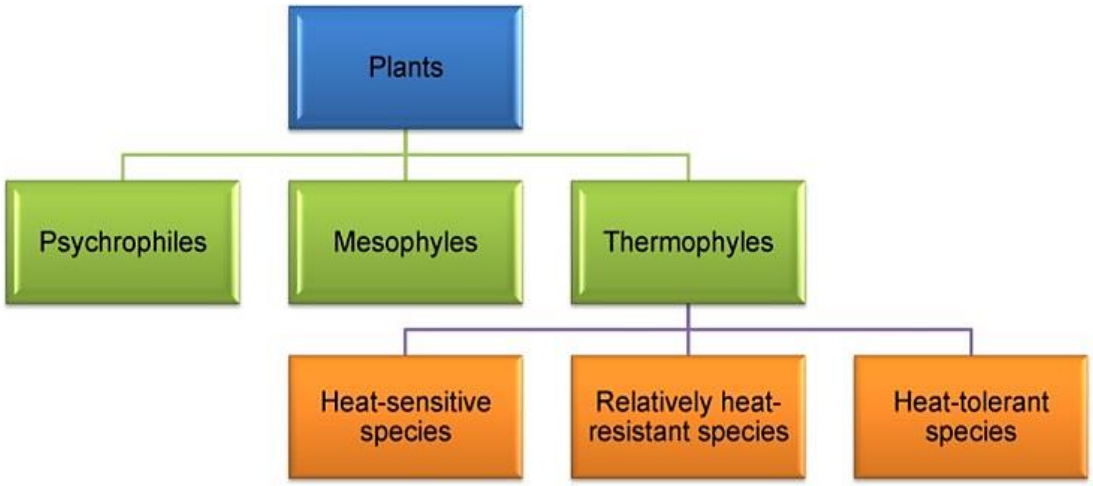

Figure 3.1 Classification of plants on the basis of their heat tolerance.

Survival in hot, dry environments can be achieved in a variety of ways, by combinations of adaptations. Plant adaptation to heat stress includes avoidance and tolerance mechanisms which employ a number of strategies (Figure 3.2).

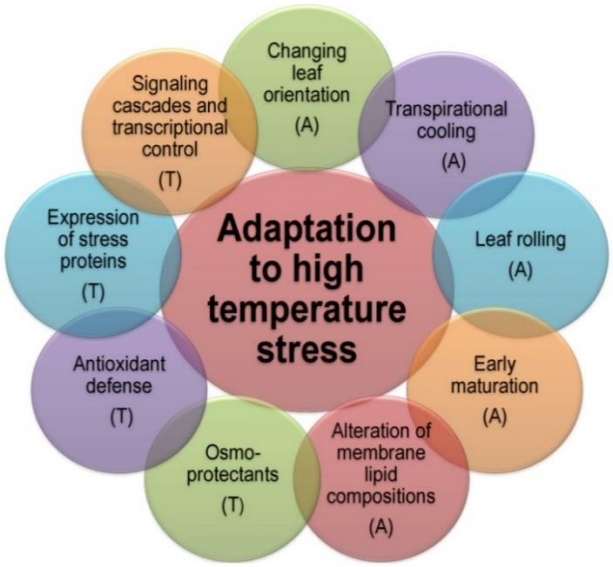

Figure 3.2 Different adaptation mechanisms of plants to high temperature.
A: Avoidance, T: Tolerance

17.3.1 Avoidance Mechanisms

Under HT conditions, plants exhibit various mechanisms for surviving which include long-term evolutionary phenological and morphological adaptations and short-term avoidance or acclimation mechanisms such as changing leaf orientation, transpirational cooling, or alteration of membrane lipid compositions. Closure of stomata and reduced water loss, increased stomatal and trichomatous densities, and larger xylem vessels are common

heat induced features in plant. In many crop plants, early maturation is closely correlated with smaller yield losses under HT, which may be attributed to the engagement of an escape mechanism. Plants growing in a hot climate avoid heat stress by reducing the absorption of solar radiation. This ability is supported by the presence of small hairs (tomentose) that form a thick coat on the surface of the leaf as well as cuticles, protective waxy covering. In such plants, leaf blades often turn away from light and orient themselves parallel to sun rays (paraheliotropism). Solar radiation may also be reduced by rolling leaf blades. Plants with small leaves are also more likely to avoid heat stress: they evacuate heat to ambient more quickly due to smaller resistance of the air boundary layer in comparison with large leaves.

17.3.2 Tolerance Mechanisms

Heat tolerance is generally defined as the ability of the plant to grow and produce economic yield under HT. Some major tolerance mechanisms, including ion transporters, late embryogenesis abundant (LEA) proteins, osmoprotectants, antioxidant defense, and factors involved in signaling cascades and transcriptional control are essentially significant to counteract the stress effects. In case of sudden heat stress, short term response, *i.e.*, leaf orientation, transpirational cooling and changes in membrane lipid composition are more important for survival. Smaller yield losses due to early maturation in summer shows possible involvement of an escape mechanism in heat stress tolerance. Different tissues in plants show variations in terms of developmental complexity, exposure and responses towards the prevailing or applied stress types. The stress responsive mechanism is established by an initial stress signal that may be in the form of ionic and osmotic effect or changes in the membrane fluidity. This helps to re-establish homeostasis and to protect and repair damaged proteins and membranes.

17.3.3 Antioxidat defence in response to heat-induced oxidative stress

Plants must be protected from heat-induced oxidative stress so that they can survive under HT. Tolerance to HT stress in crop plants has been associated with an increase in antioxidative capacity. Tolerant plants entail a tendency of protection against the damaging effects of ROS with the synthesis of various enzymatic and nonenzymatic ROS scavenging and detoxification systems. Activities of different antioxidant enzymes are temperature sensitive and activation occurs at different temperature ranges but the activities of these enzymes increase with increasing temperature. Catalase (CAT), ascorbate peroxidase (APX) and superoxide dismutase (SOD) showed an initial increase before declining at 50 °C, while peroxidase (POX) and glutathione reductase (GR) activities declined at all temperatures ranging from 20 to 50 °C. In addition, total antioxidant activity was at a maximum at 35–40 °C in the tolerant varieties and at 30 °C in the susceptible ones. Antioxidant metabolites like AsA, GSH, tocopherol and carotene also protect plants against oxidative stress.

17.3.4 Mechanism of signal transduction and development of heat tolerance

Upregulation of many genes has been reported to help the plant to withstand the stress conditions which leads to plant adaptation. Upon stress plants perceive the external and internal signals through different independent or interlinked pathways which are used to regulate various responses for its tolerance development (Figure 3.3). To generate response in specific cellular compartments or tissues against a certain stimuli, interaction of cofactors and signaling molecules are required. Signaling molecules are involved in activation of stress responsive genes. There are various signal transduction molecules related to stress responsive

gene activation depending upon plant type, types of stresses. Some broad group of those are the Ca-dependent protein kinases (CDPKs), mitogen-activated protein kinase (MAPK/MPKs), NO, sugar (as signaling molecule), phytohormones. These molecules together with transcriptional factors activate stress responsive genes. Once the stress responsive genes activate, these help to detoxify the ROS (by activating detoxifying enzymes, free radical scavengers); reactivate the essential enzymes and structural proteins and all the above stated processes help to maintain the cellular homeostasis.

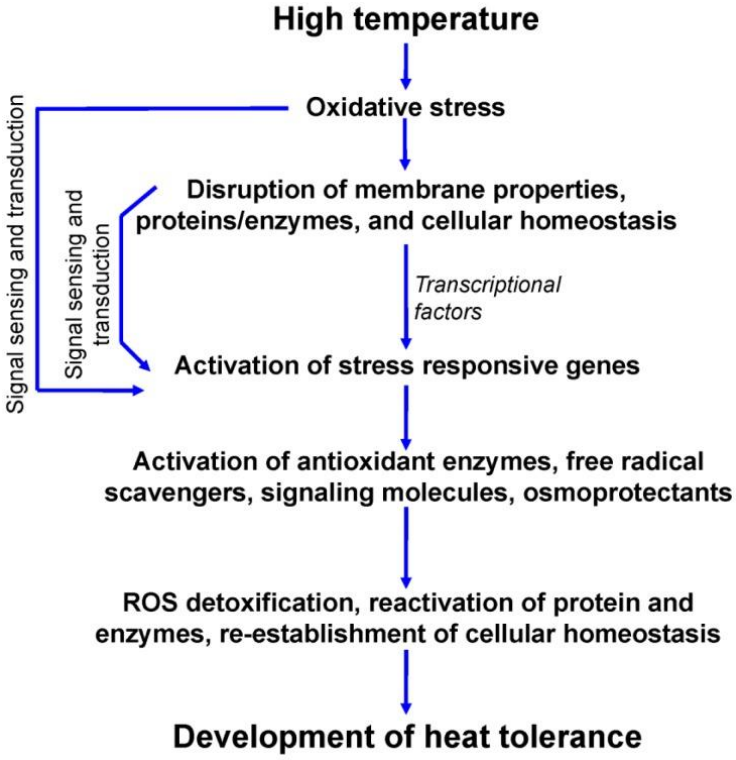

Figure 17.3 Schematic illustration of heat induced signal transduction mechanism and development of heat tolerance in plants

17.3.5 Heat-Shock Proteins (HSPs)

In general, heat stress is responsible for the up-regulation of several heat inducible genes, commonly referred as “heat shock genes” (HSGs) which encode HSPs and these active products are very much necessary for plant’s survival under fatal HT. High temperature induced constitutive expression of most of these proteins protect intracellular proteins from being denaturation and preserve their stability and function through protein folding; thus it acts as chaperones. The expression of HSPs is restricted to certain developmental stages of plant like seed germination, embryogenesis, microsporogenesis and fruit maturation. In plants, well-characterized HSPs can be grouped into five different families: HSP100 (or ClpB), HSP90, HSP70 (or DnaK), HSP60 (or GroE) and HSP 20 (or small HSP, sHSP). The HSP70 and HSP60 proteins are among the most highly conserved proteins in nature, consistent with a fundamental role in response to heat stress. Plants also have the highest diversity of sHSPs which have very low molecular mass of 12–40 kDa. For better understanding, Table 2 presents the primary molecular functions of major HSPs for heat stress tolerance in plants.

Table 2. An outline of basic function of major classes of heat shock proteins in plant system for heat stress tolerance

Major classes of heat shock protein	**Functions**
HSP100	ATP-dependent dissociation and degradation of aggregate protein
HSP90	Co-regulator of heat stress linked signal transduction complexes and manages protein folding. It requires ATP for its function
HSP70, HSP40	Primary stabilization of newly formed proteins, ATP-dependent binding and release
HSP60, HSP10	ATP-dependent specialized folding machinery
HSP20 or small HSP (sHSP)	Formation of high molecular weight oligomeric complexes which serve as cellular matrix for stabilization of unfolded proteins. HSP100, HSP70 and HSP40 are needed for its release

17.3.6 Molecular regulatory mechanism of heat shock proteins

Upon heat stress perceived by the plant cell, (**a**) monomeric heat shock factors (HSFs) are entering into the nucleus; (**b**) from the cytoplasm. In the nucleus, HSF monomers are form active trimer; (**c**) that will bind; (**d**) to the specific genomic region (promoter or heat shock element, HSE) of the respective heat shock gene (HSG). Molecular dissection of the HSF binding region of HSE showing that it is consists of one DNA binding domain and two domains for trimerization of HSFs. Successful transcription (**e**) translation and post-translational modification (**f**) lead to produce functional HSP to protect the plant cell and responsible for heat stress tolerance (Figure 3.4).

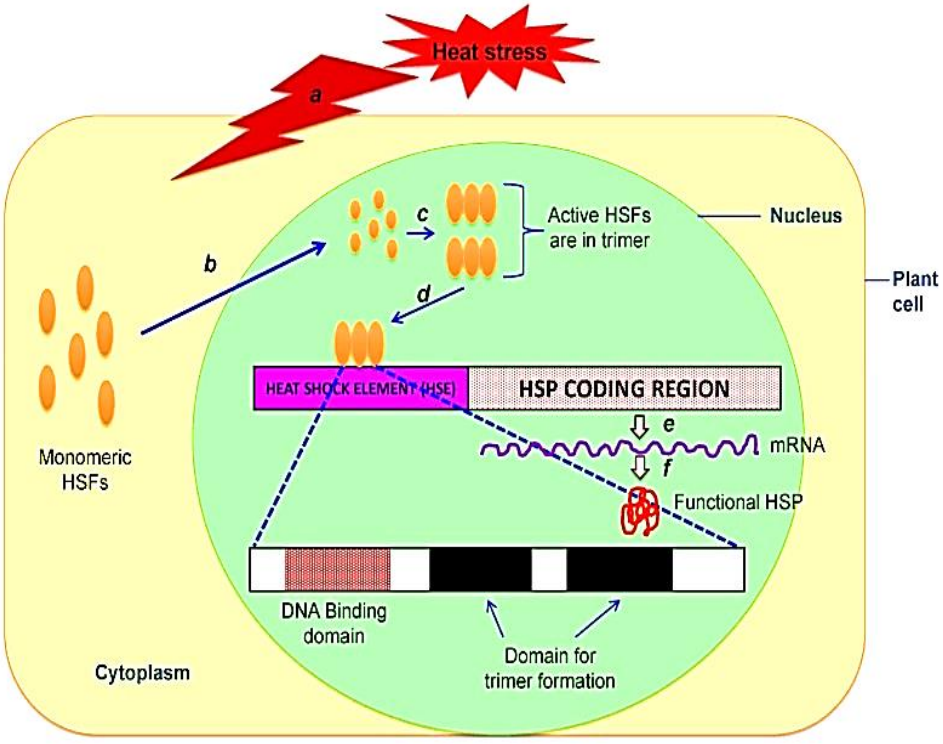

Figure 17.4 Schematic diagram showing the molecular regulatory mechanism of heat shock proteins based on a hypothetical cellular model.

17.4 LOW TEMPERATURE STRESS

Low-temperature (LT) stress is one of the abiotic stresses in plants that affect cell survival, cell division, photosynthesis, and water transport with a negative effect on plant growth, eventually constraining crop productivity. LT stress is categorized as, (i) chilling stress - low temperature (0–15°C) causes injury without ice crystal formation in plant tissues, and (ii) freezing stress (<0°C) - ice formation occurs within plant tissues. Both stresses are termed low temperature or cold stress. In general, plants originating from tropical and subtropical regions are sensitive to LT, whereas temperate plants showed chilling tolerance to variable degrees. Low temperature negatively impacts plants, may affect the survival rate of crop plants, and also affect various processes including cell division, photosynthesis, plant growth, development, metabolism, and finally reduce the yield of crop plants, especially in the tropics and subtropics. To overcome stress generated by LT exposure, plants trigger a cascade of events that enhance their tolerance by changes in gene expression and activation of the ROS scavenging system and thus inducing biochemical and physiological modifications.

17.4.1 Morpho-physiological changes in crop plants in response to LT stress

Various metabolic reactions were inhibited by LT exposure, consequently preventing the plant's full genetic expression potential expressed by diverse phenotypic symptoms. Low temperature is a limiting factor for seed germination and plant growth. Low temperatures also increased the mortality percentage of seedlings. LT dramatically affects photosynthesis as well. Under LT stress, photosynthesis is impaired, resulting in a lower amount of carbohydrates for grain production and reducing growth, adding to indirect yield loss. Low temperature decreases photosynthesis due to partial stomatal closure, slowdown of electron transport, inhibits metabolism of carbohydrates, and interferes with phloem loading. In plants, the content of both total Chl and chlorophyll b (Chl b) decreased and the Chl a/b ratio increased under low night temperature stress. Low night temperature probably enhanced the activity of chlorophyllase enzyme in leaves and hence resulted in reduced Chl synthesis. In cold-acclimated winter annuals, Calvin cycle enzymes accumulate in higher amounts and effectively maintain the photosynthetic activity of plants.

17.4.2 Oxidative stress

Plants exposed to LT stress undergo various metabolic and physiological changes and chilling stress ultimately leads to oxidative stress in plants, a physiological condition, where an imbalance occurs between the generation of reactive oxygen species and their metabolism via enzymatic and nonenzymatic antioxidants. Different types of reactive oxygen species (ROS) are accumulated under LT stress, which includes (a) singlet oxygen ($^1O_2$), (b) superoxide radical ($O_2^{\bullet -}$), (c) hydrogen peroxide (H_2O_2), and (d) hydroxyl radical ($OH^{\bullet}$). In plant cells, ROS are continuously produced as a consequence of aerobic metabolism in all the intracellular organelles, particularly in the chloroplast, mitochondria, and peroxisomes. Chloroplast is considered the main source of ROS in plants. Other ROS-producing sources include NADPH oxidases, cell wall-bound peroxidases, and amine oxidases (Figure 3.5).

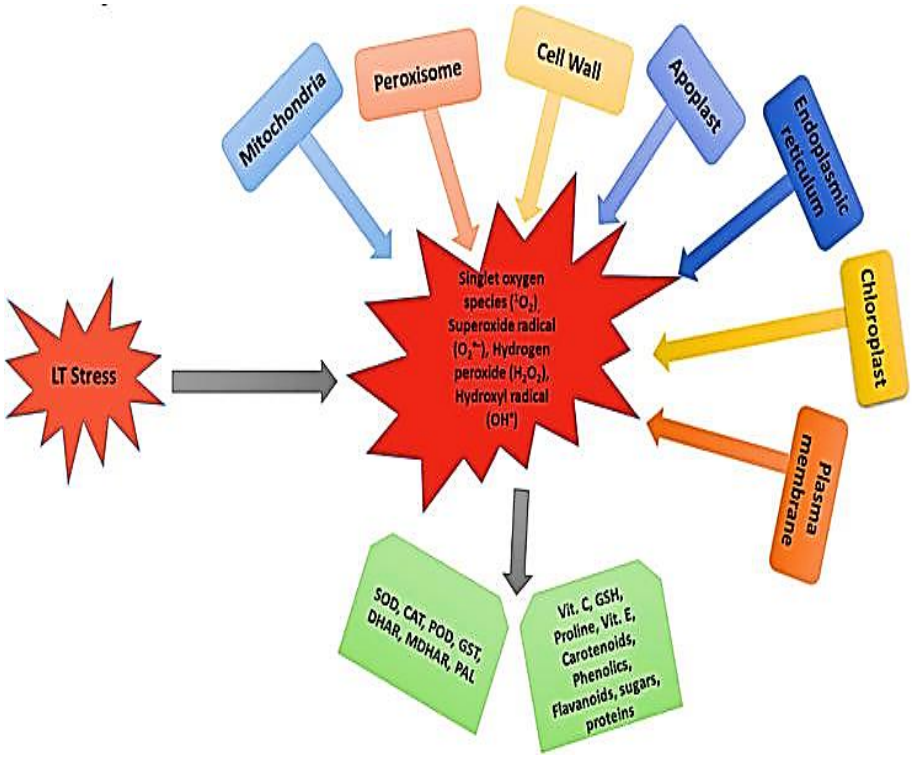

Figure 17.5 Overview of oxidative stress, production of reactive oxygen species, and its scavenging

17.4.3 Other biochemical changes

Under normal physiological conditions, ROS levels are maintained low by the action of various enzymatic and nonenzymatic antioxidants, such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), glutathione (GSH), and vitamin C. Accumulation of ROS accelerated under extremely cold conditions, beyond the plant's tolerant level due to less activity of antioxidant enzymes, which are responsible for detoxification of ROS. Higher content of ROS causes oxidative stress which is manifested as peroxidation of membrane lipids, damage to proteins, carbohydrates, and DNA, etc. ROS alters the activities of enzymes and affects various biochemical reactions and physiological processes, including nutrient movements, respiration, photosynthesis, and transpiration, thus having a negative impact on a plant's survival percentage. Membranes are a primary site of cold-induced injury because of their central role in the regulation of various cellular processes. LT stress leads to the destruction of cell membrane structure, change the permeability of membranes, and causes leakage of cell electrolytes and thus damages the plants. It has been demonstrated that LT responses are triggered by membrane rigidification, coupled with calcium influxes, cytoskeletal rearrangements, and the activation of MAPK cascades.

17.5 COLD STRESS TOLERANCE MECHANISMS

Plants have developed various tolerance mechanisms to cope up with cold stress, these includes antioxidants, cold acclimation, molecular regulation and phytohormones (Figure 3.6).

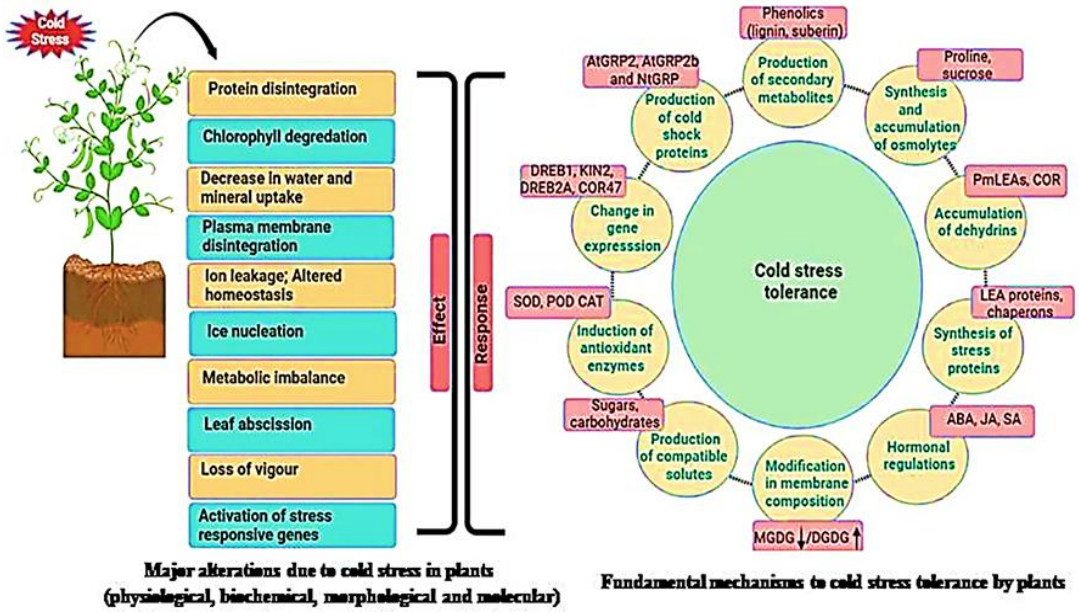

Figure17.6 Cold stress tolerance mechanisms of plants

17.5.1 Enzymatic antioxidants

Plants have developed ROS scavenging mechanisms, which include a variety of nonenzymatic and enzymatic defense systems to protect cellular membranes and organelles from the damaging effects of ROS. Types of antioxidants produced in the plants are represented in Table 1. The degree of damage by ROS depends on the balance between the accumulation of ROS products and their detoxification by the antioxidant scavenging system.

Enzymatic Antioxidants: Enzymatic antioxidants includes catalase (CAT), ascorbate peroxidase (APX), superoxide dismutase (SOD), glutathione peroxidase (GPX), glutathione reductase (GR), glutathione S-transferase (GST), dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDHAR), etc. The efficiency of the antioxidant defense system to scavenge ROS largely decides the plant's sensitivity to chilling. A higher amount of H_2O_2 produced during stress is detoxified by APX, POD, and CAT in different organelles. Catalase converts H_2O_2 into O_2 and water. CAT and POD are important enzymes that scavenge H_2O_2. There is a positive correlation between stress tolerance and the activity of POD, CAT, and SOD enzymes in plants. Higher POD and SOD activity probably suggest their possible role in mitigating adverse environmental damage.

Nonenzymatic antioxidants: The nonenzymatic antioxidants includes ascorbic acid (Vitamin C), α-tocopherol (Vitamin E), glutathione (GSH), carotenoids, phenolics, and flavonoids, etc. Nonenzymatic antioxidants abundantly present and participate in ROS scavenging. The tripeptide glutathione (γ-glutamyl-cysteinyl-glycine) is widely distributed in plant cells and is implicated in the adaptation of plants to environmental stresses, such as extreme temperatures. It is an important antioxidant associated with the regeneration of AsA in the ascorbate-glutathione cycle and participates in the removal of H_2O_2. Its antioxidant activity is mainly due to its redox buffer property. It functions to remove toxic peroxides formed in the cell during normal and stressed conditions.

17.5.2 Cold acclimation

Acclimation may be defined as changes that occur in a plant in response to chilling temperatures, which confer subsequent tolerance to the cold injury, especially during

germination and early seedling growth. Cold priming/acclimation is associated with multiple physiological and biochemical alterations, including membrane stabilization, increased ROS and methylglyoxal (MG) detoxifications, activation of cold-sensitive protein kinases, NO and hormone biosynthesis, and accumulation of antioxidants, HSPs, cold-regulated proteins (CORs), and dehydrins. Cold acclimation makes plants capable of protecting themselves from freezing-induced injury. Cold acclimation increased the abundance of ROS-scavenging proteins, LEA/RAB proteins, and dehydrins. The activity of cold/chilling-induced genes may facilitate the metabolic changes that confer LT tolerance. Cold acclimation causes the synthesis of protective molecules, such as soluble sugars, sugar alcohols, proline, and glycine betaine. These molecules in conjunction with various proteins play a role to stabilize both phospholipids and proteins of the membranes and proteins of cytoplasm, maintain hydrophobic interactions between molecules and scavenge various types of ROS, which are produced under LT. Some plants respond to LT by the synthesis of some specific proteins that are similar to plant pathogen-related (PR) proteins in response to cold and drought Eg: winter rye.

17.5.3 Molecular regulatory mechanisms for plant response to low-temperature stress

The molecular response to low-temperature stress is divided into three phases: perception and decoding of low-temperature signals, the transmission of low-temperature signals to cells, and stress adaptation of response gene expression profiles. Response signals are decoded and converted into biological signals, activating plant defence mechanisms to resist low-temperature stress. Studies in plant signal responses have established that these complex cold signalling systems and gene regulation mechanisms can be termed as ABA-dependent and -independent signalling pathway.

ABA-Independent Cold Stress Signalling Pathway in Plants

Low temperature is sensed at the plasma membrane, triggering Ca^{2+} influx. This activates calcium-binding proteins and MAPK cascades (MEKK1 → MKK2 → MPK4/MPK3/6) (Figure 3.7). ICE1/ICE2 (Inducers of CBF Expression) are basic helix-loop-helix transcription factors that activate CBF/DREB1 genes. Under cold stress, post-translational regulation of ICE is crucial: HOS1 promotes ICE degradation via ubiquitination (negative regulation). SIZ1 (SUMO E3 ligase) stabilizes ICE via sumoylation (positive regulation). ICEs activate CBF (C-repeat Binding Factor) genes (also called DREB1s). CBF proteins bind to the CRT/DRE elements in the promoters of downstream cold-responsive (COR) genes. COR genes encode proteins that protect plant cells from freezing damage (e.g., antifreeze proteins, dehydrins, LEA proteins, osmoprotectants). This leads to enhanced cold tolerance and survival.

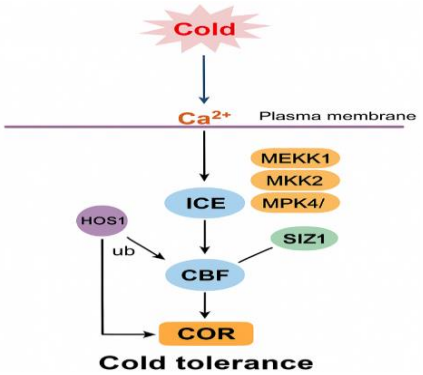

Figure 17.7 ABA-Independent Cold Stress Signalling Pathway in Plants

ABA-Dependent Molecular Pathways in Cold Stress

The abscisic acid (ABA)-dependent pathway is a central signaling mechanism by which plants respond to low temperature stress (Figure 1). Upon exposure to cold, plants accumulate ABA, which acts as a secondary messenger to regulate stress-responsive gene expression. Low temperature alters membrane fluidity, triggering calcium influx and ROS signaling. This induces ABA biosynthesis, mainly via NCED (9-cis-epoxycarotenoid dioxygenase). The increased ABA levels serve as a signal to activate downstream regulatory networks. ABA binds to PYR/PYL/RCAR receptors, which in turn inhibit PP2C (Protein Phosphatase 2C). Inhibition of PP2C activates SnRK2 (Sucrose Non-Fermenting 1-related protein kinases 2). Activated SnRK2s phosphorylate multiple transcription factors, particularly AREB/ABFs (ABA-responsive element binding proteins/factors). AREB/ABFs bind to ABA-responsive elements (ABREs) present in promoters of ABA-regulated cold-responsive genes. These transcription factors regulate expression of genes involved in osmolyte synthesis, antioxidant defense, and protective proteins. ABA signaling interacts with OST1 (Open Stomata 1) kinase, which also phosphorylates ICE transcription factors to stabilize them and indirectly promote CBF expression. Thus, ABA signaling can cross-talk with the ABA-independent ICE–CBF–COR module. ABA-induced genes encode LEA proteins, dehydrins, antifreeze proteins, osmoprotectants (e.g., proline, sugars), and antioxidant enzymes. These molecules protect cells by stabilizing membranes, preventing ice crystal formation, and maintaining osmotic balance under freezing stress.

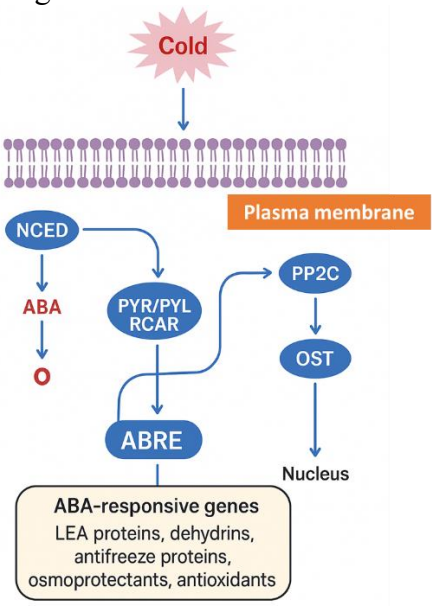

Figure 3.8 ABA-dependent pathway

17.5.4 Contribution of Phytohormones during the Cold Stress Response

Abscisic acid (ABA) is a bonafide stress hormone that plays key functions during stress. During stress conditions, carotenoids are converted into bioactive ABA by de novo biosynthesis pathway. ABA biosynthesis is increased during cold stress, which improves plant ability to resist during unfavorable cold conditions. ABA biosynthesis and catabolic genes display their regulation in organ dependent manner during cold stress. Another phytohormone, auxin (indole-3-acetic acid, IAA) has been established to control virtually all aspects of plant development and response. Emerging evidences indicate the involvement of auxin in regulation of plant growth and development under high as well as low temperature stresses. GH3 genes encode auxin-conjugating enzyme and modulate endogenous levels of

active auxin through negative feedback regulation. Recently, the involvement of auxin has been shown to preserve the root stem cells at a quiescent status under chilling stress. Phytohormones cytokinin signal transduction is mediated by the two-component system (TCS), which is perceived by histidine kinase receptors and regulate the phosphorylation of response regulators. Moreover, Cytokinin Response Factors (CRF2) and CRF3 are involved in lateral root formation and initiation during cold stress whereas, CRF4 gets induced during cold and contributes to freezing tolerance. Ethylene mediates physiological, developmental and stress responses through the activation of Ethylene Response Factors (ERFs) transcription factor. Recently, ERF105 has been shown to operate in conjunction with the CBF pathway and play a key role during cold acclimation and freezing tolerance. Additionally, high ethylene content increases freezing tolerance in non-acclimated plants. Brassinosteroids (BRs) are another class of phytohormones that play key role in plant development and defense. BRs regulate the expression of several COR genes and CBF regulation, thereby control the freezing tolerance. Besides, other phytohormones such as Gibberellic acid (GA), Salicylic acid (SA), and Jasmonic acid (JA) also contribute significantly to cold stress response.

17.6 SUMMARY

Temperature stress, encompassing both heat stress and cold stress, is a major abiotic factor that adversely affects plant growth, development, and productivity. High temperatures disrupt cellular homeostasis by causing protein denaturation, membrane destabilization, and excessive generation of reactive oxygen species (ROS), which impair photosynthesis, respiration, and reproductive processes. Heat stress also accelerates transpiration and leads to reduced pollen viability and seed set. Conversely, low temperatures (chilling or freezing stress) restrict enzymatic activities, decrease membrane fluidity, impair water transport, and induce ice crystal formation in tissues, leading to cellular dehydration and structural damage. Plants respond to temperature extremes through physiological adjustments (stomatal regulation, osmotic balance), biochemical mechanisms (antioxidant defense, accumulation of compatible solutes), and molecular responses (upregulation of heat shock proteins, dehydrins, and stress-responsive transcription factors). While short-term exposure can cause reversible damage, prolonged or severe stress results in growth inhibition, yield reduction, and, in extreme cases, plant death. Understanding plant responses to temperature stress is crucial for developing climate-resilient crops through genetic improvement and microbial/biotechnological interventions.

Cold stress, defined as exposure of plants to low but non-freezing temperatures (0–15 °C), significantly impairs growth and metabolism, particularly in cold-sensitive tropical and subtropical species. Low temperatures reduce membrane fluidity, disrupt water transport, and restrict the activity of key enzymes involved in photosynthesis and respiration. As a result, plants exhibit decreased chlorophyll content, impaired photosystem II efficiency, and reduced carbon assimilation. Cold stress also enhances the generation of reactive oxygen species (ROS), leading to oxidative damage of lipids, proteins, and nucleic acids. Physiological symptoms commonly include leaf chlorosis, necrosis, poor germination, stunted growth, and delayed flowering. To counteract these effects, plants activate adaptive responses such as accumulation of osmolytes (proline, sugars), upregulation of antioxidant defense enzymes (SOD, CAT, APX), and induction of cold-responsive (COR) genes regulated by C-repeat binding factors (CBFs). While some temperate plants naturally acclimate through membrane lipid remodeling and metabolic adjustments, cold-sensitive crops often show substantial yield

losses under low-temperature stress, making cold tolerance a key target for breeding and biotechnological interventions.

17.7 SELF-ASSESSMENT QUESTIONS

1. Define temperature stress in plants and differentiate between heat stress and cold stress.
2. What are heat shock proteins (HSPs) and what role do they play in heat tolerance?
3. Explain the term cold acclimation in plants.
4. How does temperature stress affect photosystem II (PSII)?
5. What is meant by thermopriming?
6. Mention two physiological symptoms of plants under heat stress.
7. Describe in detail the physiological, biochemical, and molecular responses of plants to heat stress.
8. Explain the role of reactive oxygen species (ROS) in temperature stress signaling and damage.
9. Describe the role of heat shock proteins (HSPs) and dehydrins in imparting tolerance to temperature extremes.
10. Explain how membrane fluidity is altered under cold stress and its consequences for plant metabolism.
11. Illustrate the molecular signaling pathways activated during heat and cold stress responses.
12. How do plants use osmolytes (e.g., proline, sugars) to mitigate temperature-induced damage?
13. Discuss the biotechnological and microbial approaches for improving plant tolerance to temperature stress.

17.8 SUGGESTED READINGS

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