جامعة بغداد

كلية العلوم

قسم التقنيات الاحيائية

فسلجة نبات/ عملي

المرحلة الثانية- الفصل الدراسي الثاني

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LAB 1----------------------ORGANIZATIONS

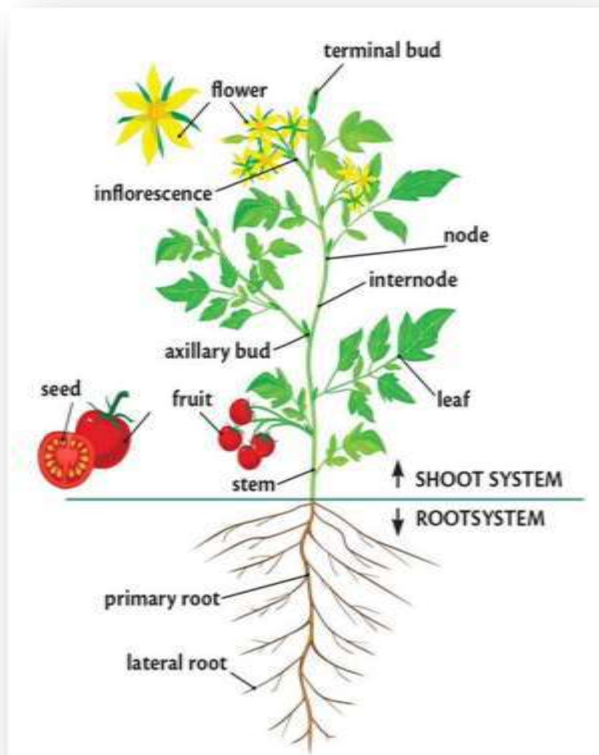
The plant has two organ systems:

1- The shoot system**.**

2- The root system.

The shoot system: Is above ground and includes the organs such as leaves, buds, stems, flowers (if the plants have any) and fruit (if the plants have any).

The root system: Includes those parts of the plant below the ground, such as the roots, tubers and rhizomes.

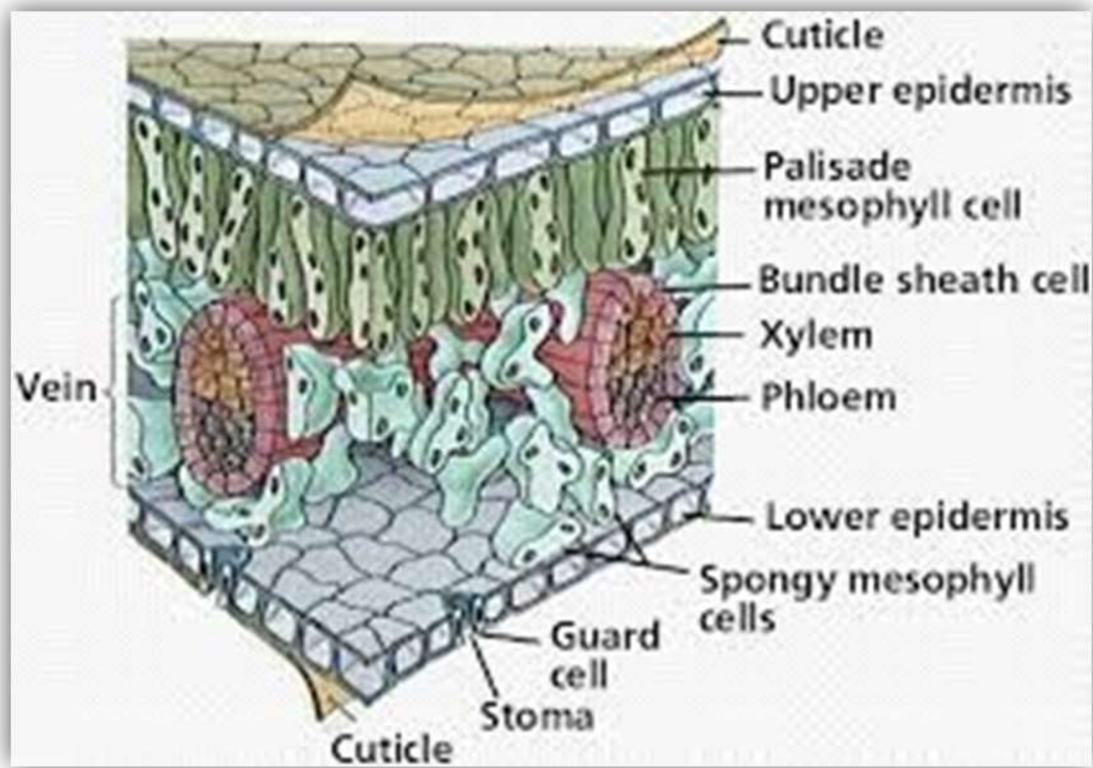


The whole plant

Plant cells are formed at meristems, and then develop into cell types which are grouped into tissues. Plants have only three tissue types:

1- Dermal 2- Ground 3- Vascular

Dermal tissue covers the outer surface of herbaceous plants. It is composed of epidermal cells, closely packed cells that secrete a waxy cuticle that aids in the prevention of water loss. The **ground** tissue comprises the bulk of the primary plant body. Parenchyma, collenchyma and sclerenchyma cells are common in the ground tissue. **Vascular** tissue transport food, hormones and minerals within the plant. Vascular tissue includes parenchyma, xylem, phloem and cambium cells.



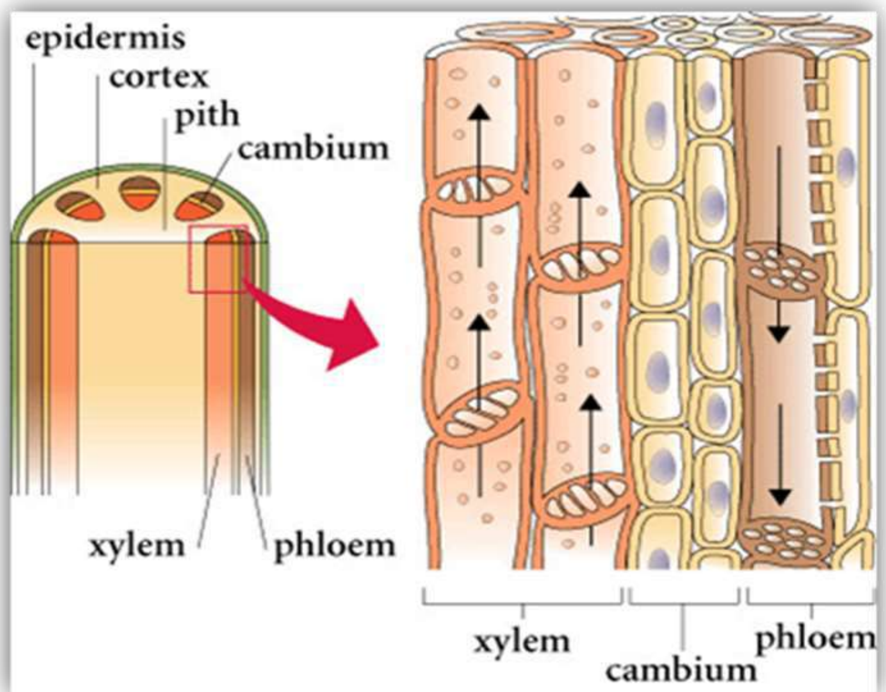
Section in a leaf

Parenchyma:

A generalized plant cell type, parenchyma cells are alive at maturity. They function in storage photosynthesis and as the bulk of ground and vascular tissue. Palisade parenchyma cells are elongated cells, located in many leaves just below the epidermal tissues.

Xylem:

It is the more identifiable cells, tracheid and vessel elements, tend to stain red with Safranian-O. Tracheids are the more primitive of the two cell types, occurring in the earliest vascular plants. Tracheids are long and tapered, with angled end-plates that connect cell to cell. Vessel elements are shorter, much wider and lack end plates. They occur only in angiosperms, the most recently evolved large group of plants. Conducting cells of the xylem; tracheids are more primitive, while the various types of vessels are more advanced.

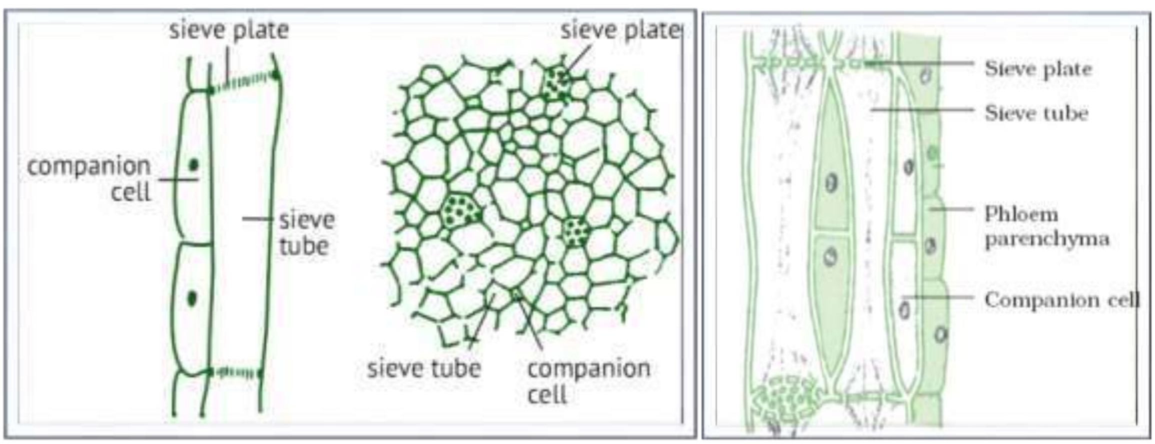


Xylem Structure

Phloem

Phloem cells conduct food from leaves to rest of the plant. They are alive at maturity and tend to stain green. Phloem cells are usually located outside the xylem. The two most common cells in the phloem are the companion cells and sieve plate. Companion cells retain their nucleus and control the adjacent sieve plate. Dissolved food, as sucrose, flows through the sieve plates.

Note the longitudinal view of the sieve plate inside the large sieve tube.



Phloem Structure

https://www.youtube.com/watch?v=_-ROXGqBSxI&t=20s root

https://www.youtube.com/watch?v=ulqN28D8ohg shoot

https://youtu.be/MxQm7KDvpDo?t=18 mangrove

LAB 2 -------------- *The solutions*

TERMS ABOUT SOLUTIONS:

3- Solution: Homogeneous mixture**.**
4- Solute: The substance that is dissolved (small amount).
5- Solvent: The dissolving substance (large amount).
6- Solvation: The interactions between the solute and solvent.
7- Hydration: The interactions between the solute and water.

TYPES OF SOLUTIONS:

There are three types of solutions depended on:

- **Size of its particles as follows:**

1- True solution (diameter of particles < 1nm).
2- Colloidal (diameter of particles 1-200 nm).
3- Suspension (diameter of particles > 200 nm).

- **Electrical conductivity as follows:**

1- High conductivity (Strong electrolytes, 100% dissociation like NaCl).
2- Slight conductivity (Weak electrolytes, partial dissociation like CH_3COOH).
3- No conductivity (Non- electrolytes, no- dissociation like sugar).

- **Saturated solutions and solubility:**

1- **Unsaturated solution**: There are fewer particles or solutes present than solvent in the solution.
2- **Saturated solution**: When no more solvent can be dissolved.
3- **Super saturated solution**: Solution contains more solute than in necessary to form a saturated solution. Super solution is formed by making a saturated solution then slowly cooling it.

PROPERTIES OF COLLOIDAL SOLUTION:

1- **Filter ability**: The colloidal solution cannot be filtered.

2-Electric properties: All colloidal particles in a colloidal solution carry electric charge of the same sign. As a result, they repel each other and remain dispersed in the dispersion medium.

3-Brownian movement: Colloidal particles are kept dispersed throughout the dispersing medium by random collisions.

4-Tyndall effect: The scattering of light by particles in a colloidal.

5-Precipitation: The precipitation of the colloidal particles by the addition of an electrolyte is called as flocculation.

**Types of colloidal

Solution, Gel and **Emulsion**

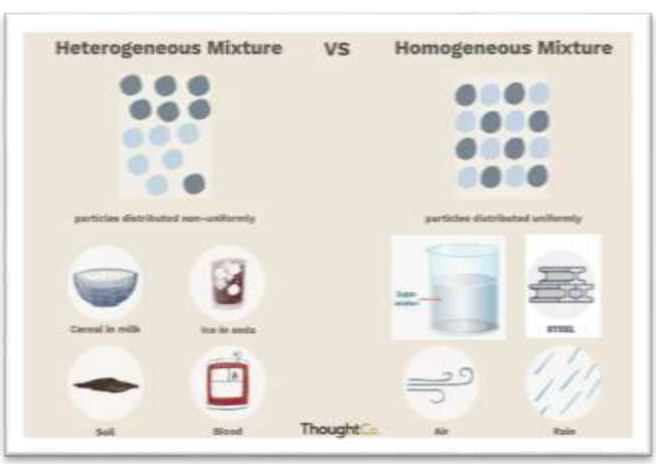
** Preparation of solutions

***True solution:** Add 5gm of NaCl to 100ml of D.W.

***Colloidal solution:** Shake 2 gm of starch with a little cold water in test tube until it form paste .Then boil 100 ml of D.W. in a beaker and while the water is boiling, slowly pour the paste from the test tube to it.

***Suspension:** Mix 2 gm of soil with 15 ml of D.W.

Note: The difference between heterogeneous and homogeneous solution; heterogeneous mixtures have visually distinguishable components, while homogeneous mixtures appear uniform throughout.



The most common type of homogenous mixture is a solution, which can be a solid, liquid, or gas.

Ways of expressing concentrations:

1- Mass percentage, ppm, ppb.

A- Mass percentage: Grams of solute per hundred grams of solution (w/w).

$$\text{Mass \% of component} = \frac{\text{Grams of solute}}{\text{Grams of solution}} * 100$$

B- The concentration of a solute is often expressed as a percentage of the total solution (w/vol).

Example: Prepare 10%solution of sucrose. The solution is prepared by dissolving 10 gm of sucrose in water for a total volume of 100 ml.

C- The concentration of the solute is often expressed as a volume of the total solution (vol/vol).

Example: Prepare 10% solution of HCl. The solution is prepared by adding 10 ml of HCl to water for a total volume of 90 ml.

D- Part per million (ppm): Grams of solute per million grams of solvent.

$$\text{Ppm} = \frac{\text{Grams of solute}}{\text{Grams of solution}} * 10^6$$

E- Part per billion (ppb): Grams of solute per billion grams of solvent.

$$\text{Ppb} = \frac{\text{Grams of solute}}{\text{Grams of solution}} * 10^9$$

2- Molarity, Molality and Normality

A- MOLARITY: Moles of solute per liter of solvent. It is one of the many ways to measure concentration of a solution; it's abbreviated with (M).

$$\text{Molarity (M)} = \frac{\text{Moles of solute}}{Volume\ (Liter) of solvent}$$

B- MOLALITY: Moles of solute per kilogram of solvent. It is an additional way to measure concentration of a solution; it's abbreviated with (m).

$$\text{Molality (m)} = \frac{\text{Moles of solute}}{Kilogram\ of\ solvent}$$

C- NORMALITY: Numbers of equivalents of substance per liter of solvent, its abbreviated with (N).

$$\text{Normality (N)} = \frac{\text{No.of equivalents}}{Volume\ (Liter)}$$

$$\text{No. of equivalents} = \frac{\text{Weight}}{Eq.\text{Weight}}$$

$$\text{Eq. wt.} = \frac{\text{M.wt.}}{Valance}$$

LAB 3 ------------- *Diffusion, Osmosis and Imbibition*

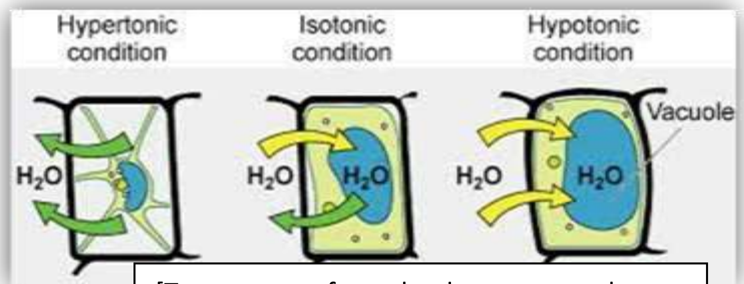
DIFFUSION: It is the net movement of substances from areas of higher concentration to areas lower concentration. The movement of substances into and out of cells (into and out of the bodies of organisms) is accomplished largely by diffusion.

OSMOSIS: It is the diffusion of water across a differentially permeable membrane. The osmosis depended on the osmotic pressure.

There are three possible conditions in regards to the concentration of water in the cell relative to its environment:

1- **ISOTONIC:** the solute concentration is the same on both sides of the cell membrane.
2- **HYPOTONIC:** the solute concentration is lower outside of the cell membrane. A cell under these conditions will tend to swell.
3- **HYPERTONIC:** the solute concentration is greater outside the cell than inside. A cell under these conditions will tend to shrink.

Very Important



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PLASMOLYSIS:

If plant cell or tissue is place in hypertonic solution, water comes out from the cell sap into the outer solution and the protoplasm begins to contract from the cell wall. This phenomenon is called **incipient plasmolysis**.

If the outer hypertonic solution is very much concentrated in comparison to the cell sap, the shrinkage of protoplasm continuous and ultimately the protoplasm separates from the cell wall. This phenomenon is called as **permanent plasmolysis**.

IMBIBITION: It is the diffusion of water into a dehydrated substrate having an affinity for water. Examples of imbibition include the expansion of the seeds doors and sponges when they absorb water.

PLANT CELLS AS OSMOTIC SYSTEMS:

Living cells in plants from osmotic systems due to the presence of semi-permeable plasma membrane and the cell sap having a certain osmotic pressure. Plasma membrane knows as selectively permeable membrane. The **tonoplast** -vacuolar membrane- (a membrane which bound the chief vacuole of a plant cell), also possesses the same nature, while the cell wall is permeable.

If a living plant cell or tissue is placed in water or hypotonic solution (which the (osmotic pressure) O.P. is lower than that of cell sap) water enters into the cell sap by osmosis. As a result of entry of the water into the cell sap, a pressure is developed which presses the protoplasm against the cell wall and the cell becomes turgid, this pressure is called a **turgor pressure**. Consequence of the turgor pressure is the wall pressure which is exerted by the elastic cell wall against the expanding protoplasm. At a given time turgor pressure **(T.P.)** equals the wall pressure **(W.P.)**

$$\boxed{\textbf{T.P.} = \textbf{W.P.}}$$

ABSORPTION OF WATER

First step in the absorption of water is the imbibition of soil water by the hydrophilic cell walls of root hairs. Osmotic pressure of the cell sap of root hairs is usually higher than the O.P. of the soil water. Therefore, water from the cell walls enters into them through plasma membrane (semi- permeable) by osmotic diffusion. As a result, the O.P. of root hairs now becomes lower, while their turgor pressure is increased.

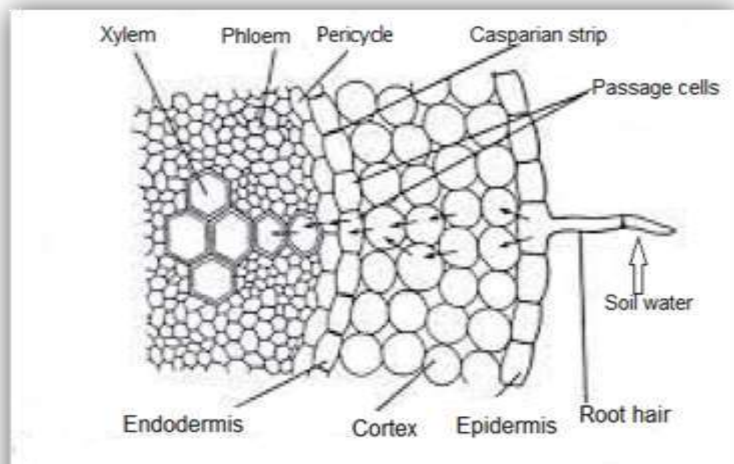
Now, the cortical cells adjacent to root hairs have higher O.P. in comparison to the root hairs. Therefore, water is drawn into the adjacent cortical cells from the root hairs by osmotic diffusion.

In the same way, the water by cell to cell osmotic diffusion gradually reaches the inner most cortical cells and the endodermis.

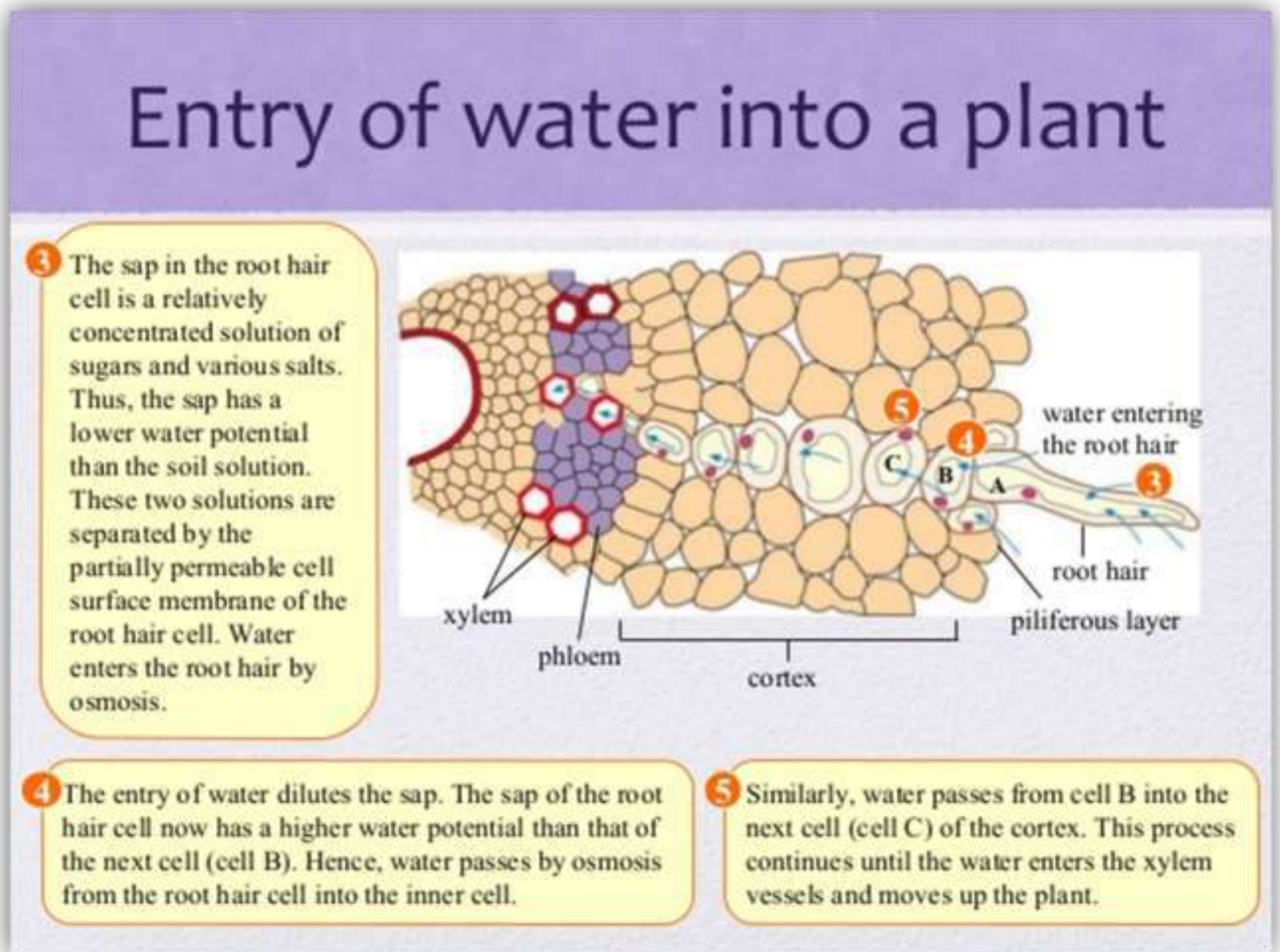
Osmotic diffusion of water into endodermis takes place through special thin walled passage cells because the other endodermal cells have casparian strips (is a band-like thickening in the center of the root endodermis (radial and cell walls) of vascular plants) on their walls which are impervious to water.

Water from endodermal cells is drawn into the cells of pericycle (a thin layer of plant tissue between the endodermis and the phloem) by osmotic diffusion which now becomes turgid and their O.P. is decreased.

In the last step, water is drawn into xylem from turgid pericycle cells. It is because in absence of turgor pressure of the xylem vessels (which are non- elastic), the O.P. of xylem vessels becomes higher than O.P. of cells of the pericycle. When water enters into xylem from pericycle, a pressure is developed in the xylem of roots which can raise the water to a certain height in the xylem. This pressure is known as **root pressure**.



Part of typical dicot root (The arrows indicate the path of water)



FACTORS EFFECTING ON THE PERMEABILITY OF THE PLASMA MEMBRANE

There are many factors that effecting on the permeability of the plasma membrane as the following:

1- Temperature
2- Alcohol
3- Freezing
4- Hypertonic solution
5- Hypotonic solution

Effect of Temperature and Alcohol on the Permeability of the Plasma Membrane

Small equal sized pieces of beet root washed with tap water. Each of these pieces is placed in a separate test tubes containing water at different temperature such as: **(0, 10, 20, 30,**

40, 60, 70 and 80^0C). One of these test tubes filled with alcohol instead of water at ordinary temperature.

After sometime it is observed that the water in those tubes which were kept at lower temperature, room temperature or slightly higher temperature, remain colorless while the water in test tubes kept in higher temperatures (e.g. **60 , 70 and 80^o C**) becomes red colures. The intensity of the colure increases with the increasing of temperature. The appearing of red colure is due to the diffusion of the **betacyanin pigments** from the cell sap into the water because at higher temperatures the semi permeability of the plasma membrane is gradually lost.

The alcohol in test tube containing pieces of beet root tissue also becomes red color because the cells are killed with alcohol (alcohol is a dehydrating agent) and the plasma membrane is destroyed so that the betacyanin pigments diffuse freely out of the dead cell and make it colored.

LAB 4 ---------------

Transpiration in Plant

Introduction

Transpiration is the evaporation of water from plants. It occurs chiefly at the leaves while their stomata are open for the passage of CO_2 and O_2 during photosynthesis.

Air that is not fully saturated with water vapor (100% relative humidity) will dry the surfaces of cells with which it comes in contact. So the photosynthesizing leaf loses substantial amount of water by evaporation. This transpired water must be replaced by the transport of more water from the soil to the leaves through the xylem of the roots and stem.

Transpiration is not simply a hazard of plant life. It is the "engine" that pulls water up from the roots to:

- **Supply** photosynthesis (1% -2% of the total).
- **Bring** minerals from the roots for biosynthesis within the leaf.
- **Cool** the leaf.

Discuss of water transport through the xylem.

Using a potometer (**right**), one can study the effect of various environmental factors on the rate of transpiration. As water is transpired or otherwise used by the plant, it is replaced from the reservoir on the right. This pushes the air bubble to the **left** providing a precise measure of the volume of water used.

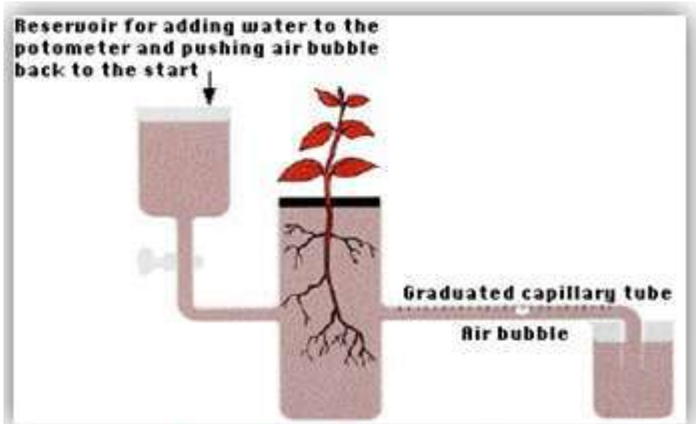


Fig.1: The Potometer

**Environmental factors that affect the rate of transpiration:

1.Light

Plants transpire more rapidly in the light than in the dark. This is largely because light stimulates the opening of the stomata (mechanism). Light also speeds up transpiration by warming the leaf.

2.Temperature

Plants transpire more rapidly at higher temperatures because water evaporates more rapidly as the temperature rises. At 30°C, a leaf may transpire three times as fast as it does at 20°C.

3.Humidity

The rate of diffusion of any substance increases as the difference in concentration of the substances in the two regions increases. When the surrounding air is dry, diffusion of water out of the leaf goes on more rapidly.

4.Wind

When there is no breeze, the air surrounding leaves become increasingly humid thus reducing the rate of transpiration. When a breeze is present, the humid air is carried away and replaced by drier air.

5. Soil water

A plant cannot continue to transpire rapidly if its water loss is not made up by replacement from the soil. When absorption of water by the roots fails to keep up with the rate of transpiration, loss of turgor occurs, and the stomata close. This immediately reduces the rate of transpiration (as well as of photosynthesis). If the loss of turgor extends to the rest of the leaf and stem, the plant wilts. The volume of water lost in transpiration can be very high.

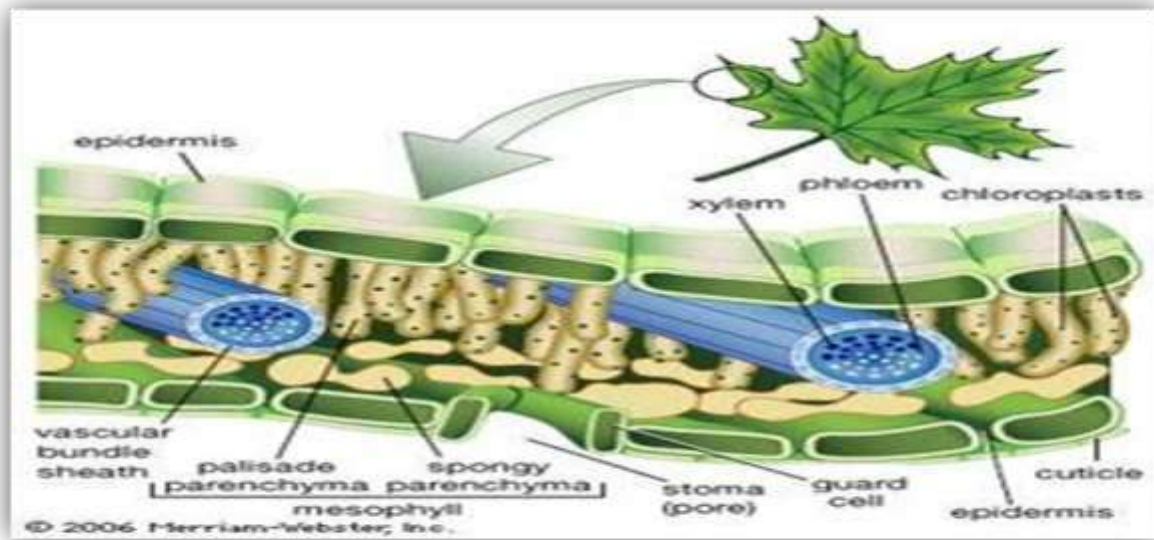


Fig.2: Section of leaf

Kinds of transpiration:

1. **Stomatal transpiration:** Most of transpiration takes place through stomata.
2. **Cuticular transpiration:** Although cuticle is impervious to water, still some water may be lost through it. It may contribute a maximum of about 10% of the total transpiration.
3. **Lenticular transpiration:** Some water may be lost by woody stems transpiration through lenticel.

**Mechanism of Stomatal Transpiration:

The Mechanism of stomatal transpiration which takes place during the day time can be studied in three steps:

1- Osmotic diffusion of water in the leaf from xylem to intracellular space above the stomata through the mesophyll cells.

2- Opening and closing of stomata.

3- Simple diffusion of water vapors from intracellular space to outer atmosphere through open stomata.

Very Important

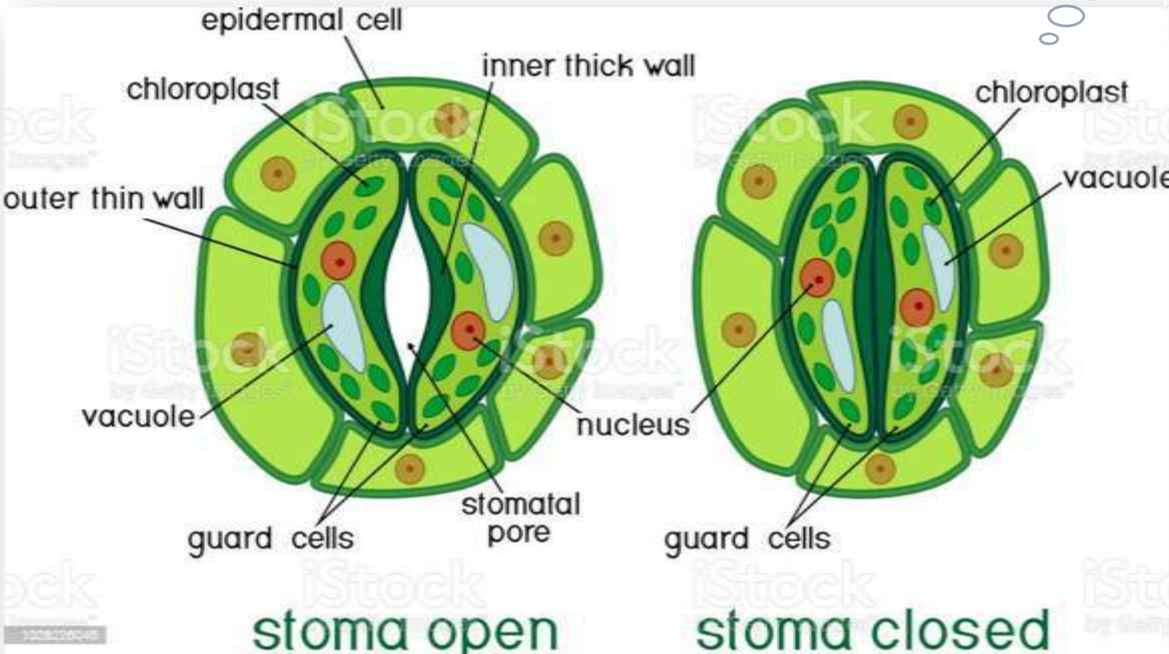


Fig.3: Opening and closing of stomata

LAB 5 ---------- *Plant pigments*

Introduction

Pigment is a molecule that absorbs and reflects light. Different pigments appear different colors because they have differing abilities to absorb and reflect various colors of light. The broad array of colors found in plant tissues such as leaves, flowers and fruits can be accounted for by the presence of literally thousands of different kinds of plant pigments.

Some types of plant pigments are present in following table:

Pigment Type	***Colors***	***Found in***
Chlorophylls	Yellow greenish- green olive	Spinach
Carotenoids	Yellow – red	carrots
Anthocyanins	Blue/purple/red	berries, grapes, red peppers, beets, eggplant, plums
Anthoxanthins	Yellow – ivory	
Betacyanins	Yellow - red/purple	Carrots, pumpkin, sweet potatoes, citrus, papaya, melon.
Xanthophylls (a subclass of carotenoids)	Ivory – yellow	Carrots, pumpkin

A) Chlorophylls:

Often hides the other pigments present in leaves. In autumn, chlorophyll breaks down, allowing xanthophyll and carotene to show their colors. Chlorophylls divided into four subclasses:

1- **Chlorophyll a:** Greenish blue in color with chemical formula $C_{55}H_{77}O_5N_4\,Mg$

2- **Chlorophyll b**: Greenish yellow in color with chemical formula $C_{55}H_{70}O_5N_4\,Mg$

3- **Chlorophyll c**

4- **Chlorophyll d**

B) Carotenoids:

1. **Carotenes** : Orang in color with chemical formula C_{40}
2. **Carotenols** : Red in color with chemical formula C_{40}
3. **Xanthophyll** : Yellow in color with chemical formula C_{40}H

C) Phycobilines:

1. **Phycoerthrin:** Red in color.
2. **Phycocyanin:** Blue in color.

Carotenoids and Phycobilines involved indirectly in photosynthesis, through:

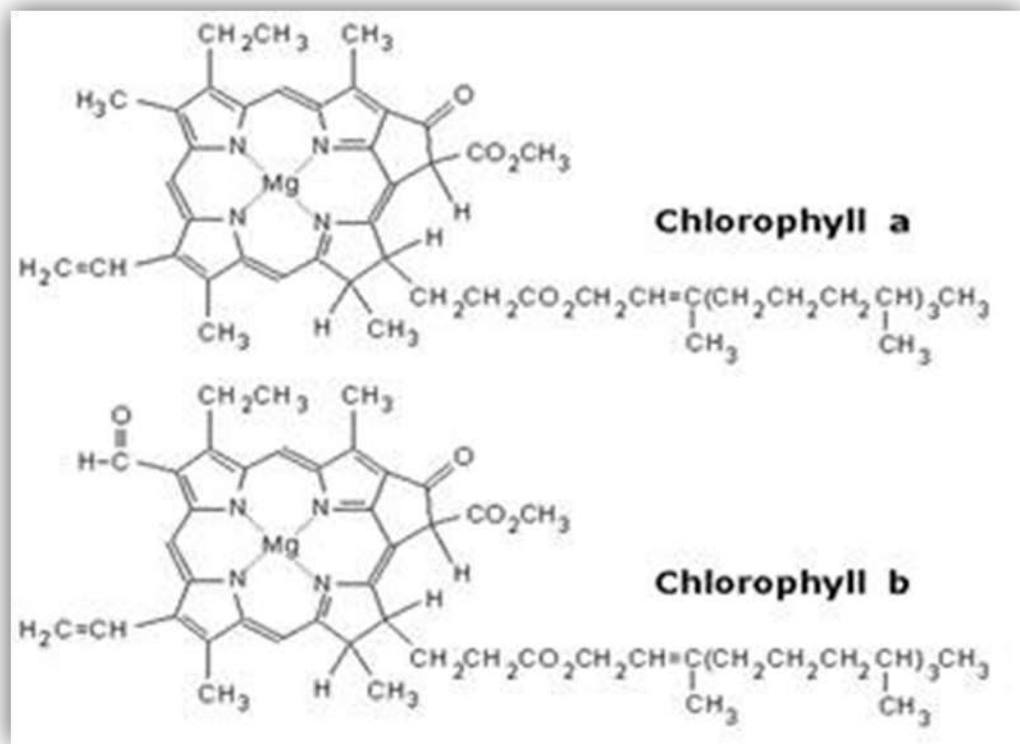
1. Protect the leaves from observed sunlight.
2. Increase the absorption spectrum of light.

Chlorophylls **a** and **b** are the main pigments involved in photosynthesis. The main differences between Chlorophylls **a** and **b** are:

Chlorophyll a	Chlorophyll b
Greenish blue in color	Greenish yellow in color
Chemical formula $C_{55}H_{77}O_5N_4\,Mg$	Chemical formula $C_{55}H_{70}O_5N_4\,Mg$
The chemical structure contains (CH_3) group.	The chemical structure contains (CHO) group.
Appeared after Chlorophyll b on paper chromatography (why?)	Appeared before Chlorophyll a on paper chromatography (why?)

The chlorophyll pigment composed of two parts:

1. **Tail (phytol)**: Consists of long chain of carbon atoms which have been attached by H and O atoms.
2. **Body (porphyrin)**: Contains Mg atom in the center of the body which is surrounded by hexagonal or pentagonal rings.



The structures of chlorophyll a and b (Chlorophyll a R=CH_3, Chlorophyll b R= CHO)

Separation of pigments

1. Take 5 gm of fresh spinach leaves after removing the midrib (why?) and place in porcelain mortar containing 10ml of 80% acetone and 2 gm of $CaCO_3$(why?).
2. Homogenize the leaves tissue gently to avoid the destruction of the plastids.
3. Filter the extract using gauze to remove the crushed leaves.
4. Place the filtrate in separating funnel containing 10 ml of ether (why?) and shake the funnel.

5. Add 10ml D.W gently on the inner surface of the funnel to avoid the formation of white emulsion layer.
6. Mix the mixture by shaking the funnel and then allow standing for appropriate time. Two layers will form in the funnel. The upper layer with petroleum ether and pigments, whereas the lower layer contains water and proteins. Discard the lower layer and place the other.
7. Place the extract on appropriate place. Try to concentrate the extract on the place drops by adding enough extract (20 drops about) at different times to ensure the dryness of the place before each addition (why?).
8. Try to conduct the experiment in a cold place to inhibit the destruction of the chlorophyll by the chlorophyllase enzyme presents in the extract.
9. Place the strip of paper chromatography in glass jar containing 10ml petroleum ether. The solution will move up on the paper by capillary action causing movement of the pigments upward to distance depend on the type of pigment.
10. After a define time, remove a paper from the jar and mark the distance of solvent flow immediately by using pencil. Allow the paper to dry then determine the distance of flow of each pigment.
11. Determine the relative flow (RF) of each pigment by the following equation:

$$RF = \frac{Distance\ of\ pigment}{Distance\ of\ solvent} < 1$$

LAB 6 ------------ *The Plant Hormones*

Introduction

Plant hormones are a group of naturally occurring, organic substances which influence physiological processes at low concentrations. The processes influenced consist mainly of growth, differentiation and development. Each has a multiplicity of effects depending on:

a- Site of action.

b- Development stage of plant.

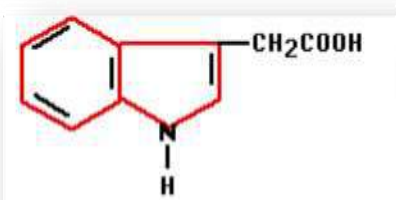
c- Concentration of hormone.

There are different classes of phytohormones:

1- Auxins
2- Cytokines (CKs)
3- Gibberellins (GA)
4- Ethylen
5- Abscisic acid (ABA)

1- Auxins:

Plant hormones produced by shoot meristems which help cause elongation and specifically for development of plant organs in the cells. The most important auxin produced by plants is **indole-3-acetic acid (IAA)**.



Indole-3-acetic acid (IAA)

Very Important

**Effects of Auxins:

* Auxins stimulate root system growth.

* Root initiation - auxin stimulates root initiation on stem cuttings, and also the development of branch roots and the differentiation of roots in tissue culture.

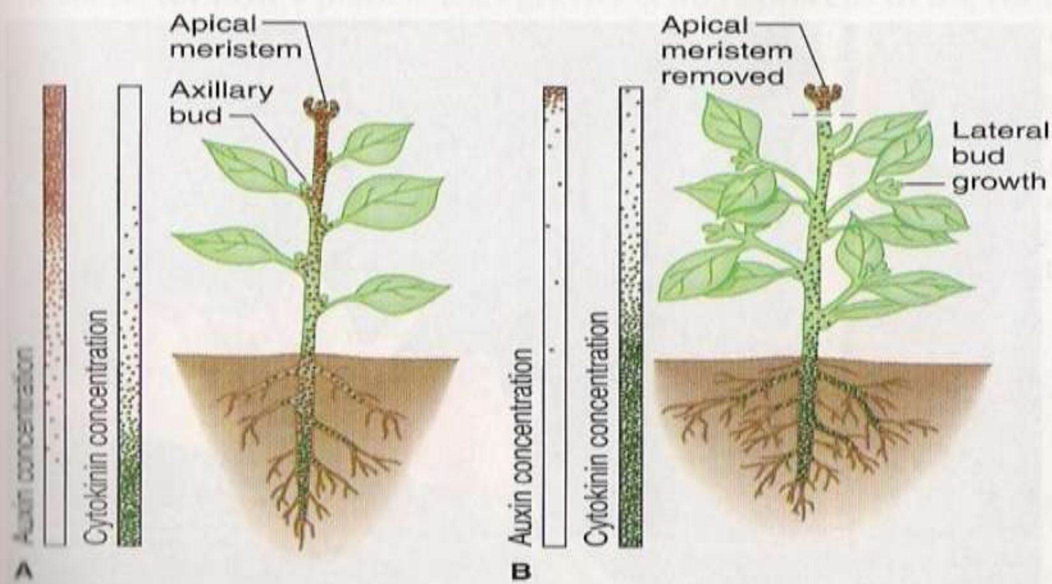
* Tropistic responses - auxin mediates the tropistic (bending) response of shoots and roots to gravity and light.

* Apical dominance - the auxin supply from the apical bud represses the growth of lateral buds.

* Delayed leaf senescence.

* Delayed fruit ripening.

FIGURE 29.4
Apical Dominance.
(**A**) High concentrations of auxin enable the apical meristem to grow at the expense of lateral (axillary) buds. (**B**) Removing the apical meristem changes the auxin-cytokinin balance, which lifts this control and stimulates the lateral buds to grow.



2- Cytokines:

Cytokines are compounds with a structure resembling adenine which promotes cell division; cytokines concentrations are highest in meristematic regions and areas of continuous growth potential such as roots, young leaves, developing fruits, and seeds. The most common cytokines base in plants is **zeatin** which was isolated from corn.

Effects:

* Cell division - applications of CKs induce cell division in tissue culture in the presence of auxin. The presence of CKs in tissues with actively dividing cells (e.g., fruits, shoot tips) indicates that CKs may naturally perform this function in the plant.

* Growth of lateral buds - CKs applications can cause the release of lateral buds from apical dominance.

* Leaf expansion - resulting solely from cell enlargement. This is probably the mechanism by which the total leaf area is adjusted to compensate for the extent of root growth, as the amount of CKs reaching the shoot will reflect the extent of the root system.

* CKs may enhance stomatal opening in some species.

* Promotes the conversion of leukoplasts into chloroplasts via stimulation of chlorophyll synthesis.

3- Gibberellin (GA)

Gibberellins (GAs) are plant hormones that regulate growth and influence various developmental processes.

Effects:

* Stimulate stem elongation by stimulating cell division and elongation.

* Stimulates bolting/flowering in response to long days.

* Breaks seed dormancy in some plants which require stratification or light to induce germination.

* Enzyme production during germination, GA stimulates the production of numerous enzymes, notably a-amylase, in germinating cereal grains.

Actions:

Before the photosynthetic apparatus develops sufficiently in the early stages of germination, the stored energy reserves of starch nourish the seedling. Usually in germination, the breakdown of starch to glucose in the endosperm begins shortly after the seed is exposed to water. Gibberellins in the seed embryo are believed to signal starch hydrolysis through inducing the synthesis of the enzyme α-amylase. In the model for gibberellin-induced production of α-amylase, it is demonstrated that gibberellins stimulate the secretion α-amylase. α- Amylase then hydrolyses starch, which is abundant in many seeds, into glucose that can be used in cellular respiration to produce energy for the seed embryo. Studies of this process have indicated gibberellins cause higher levels of transcription of the gene coding for the α-amylase enzyme, to stimulate the synthesis of α-amylase.

4- Ethylen

The gas ethylene (C_2H_4) is synthesized in many tissues in response to stress, it is synthesized in tissues undergoing synthesis of α-amylase

5-Abscisic acid (ABA)

Abscisic acid is a compound was thought to play a major role in abscission of fruits. Otherwise, induces seeds to synthesize storage proteins. Converts vegetative buds (active) to dormant buds and inhibits growth.

LAB 7 ----------- The Plant Defense Mechanisms: Part 1

(Physical Structures and Barriers)

Introduction

Plants face two types of enemies: herbivores and pathogens. Herbivores both large and small use plants as food, and actively chew them. Pathogens are agents of disease. The first line of defense in plants is an intact and impenetrable barrier composed of bark and a waxy cuticle. Both protect plants against herbivores. Other adaptations against herbivores include hard shells, thorns, and spines. They discourage animals by causing physical damage or by inducing rashes and allergic reactions. In addition, many metabolites are toxic and can even be lethal to animals that ingest them. Therefore, plants have several physical and chemical ways to defend themselves.

Following are some examples of physical means:

1- Thorns

Thorns are basically pointy branches or stems. Their defensive function is: they're stabbing.

2- Prickle

Unlike thorns, prickles are actually pointed swellings from a plant's epidermis. They mimic the spiky appearance of some planthopper.

Thorns

Prickle

3- Spine

Are modified leaves. In cacti, spines are wholly transformed leaves that protect the plant from herbivores, release heat from the stem during the day, and collect and drop condensed water vapor during the cooler night.

The spines

The main difference between spines, thorns, and prickles is:

1. **Thorns** are modified stem of a plant.
2. **Spines** are modified leaf.
3. **Prickles** are an outgrowth from the epidermis of the plant.

4- Trichomes

Have many different features that vary between both species of plants and organs of an individual plant. Some defining features include:

- Straight (upright with little to no branching), Spiral (corkscrew-shaped) or Hooked (curved apex)
- Glandular (secretory) vs. non-glandular (defense).

A macro shot of tomato trichomes

5-Cell wall

It is the outermost barrier which any pathogen has to breach for successful invasion and establishment. See the role of cell wall in lab 8.

6-Mutualism

It is the association between organisms of two different species by which each benefit. Mutualistic arrangements are most likely to develop between organisms with widely different living requirements.

The partnership between nitrogen-fixing bacteria and leguminous plants is one example.

Another example is Acacia ants inhabit the bullhorn acacia. The ants obtain food and shelter, and the acacia depends on the ants for protection from browsing animals, which the ants drive away. Neither member can survive successfully without the other, also exemplifying obligative mutualism.

Bullhorn Acacia Ants

LAB 8 -------- The Plant defense mechanisms: Part 2

(Defense responses against pathogens/chemical signals)

Pathogens are agents of disease. These infectious microorganisms, such as fungi, bacteria, and nematodes, live on the plant and damage its tissues. Plants have developed a variety of strategies to discourage or kill attackers.

A plant's exterior protection can be compromised by mechanical damage, which may provide an entry point for pathogens. If the first line of defense is breached, the plant must resort to a different set of defense mechanisms, such as toxins and enzymes.

Secondary metabolites are compounds that are not directly derived from photosynthesis and are not necessary for respiration or plant growth and development. Many metabolites are toxic and can even be lethal to animals that ingest them.

Additionally, plants have a variety of inducible defenses in the presence of pathogens. In addition to secondary metabolites, plants produce antimicrobial chemicals, antimicrobial proteins, and antimicrobial enzymes that are able to fight the pathogens.

Plants can also close stomata (**on cell wall**) to prevent the pathogen from entering the plant. A hypersensitive response (**HR**), in which the plant undergoes rapid cell death to fight off the infection, can be initiated by the plant; or it may use endophyte assistance: the roots release chemicals that attract other **beneficial bacteria** to fight the infection (e.g., rhizobia).

Mechanical wounding and predator attacks activate defense and protective mechanisms in the damaged tissue and elicit long-distancing signaling or activation of defense and protective mechanisms at sites farther from the injury location. Some defense reactions occur within minutes, while others may take several hours.

Terms you should understand and keep always in mind before we go on:

- **Biotrophic Pathogens** are plant-pathogenic fungi and bacteria which establishes a long-term feeding relationship with the **living cells** of a host, **without killing** it as part of the infection process.
- **Necrotrophic pathogens** are bacterial, fungal and oomycete species that have very destructive pathogenesis strategies resulting in **extensive necrosis**, **tissue maceration**, and plant rots.
- **Hemibiotrophic pathogens** are known to secrete a range of so-called **effector proteins**, including transcription factors and others with enzymatic activities, into host cells via the type III secretion system (T3SS) whereupon they suppress program cell death (**PCD**) and other host defenses. Usually, such kind of pathogens start their life with biotrophic lifestyle which requires a living host, then transform to a necrotrophic lifestyle which kills the host eventually.

A plant's sensory response to external stimuli relies on chemical messengers (**hormones**). Just as in animals, hormones are signaling molecules which are present in very small amounts, transported throughout the plant body, and only elicit in responses in cells which have the appropriate hormone receptors. In plants, hormones travel large distance throughout the body via the vascular tissue (xylem and phloem) and cell-to-cell via plasmodesmata.

Potentially every cell in a plant can produce plant hormones. In contrast, many animal hormones are produced only in specific glands. Plants do not have specialized hormone-producing glands.

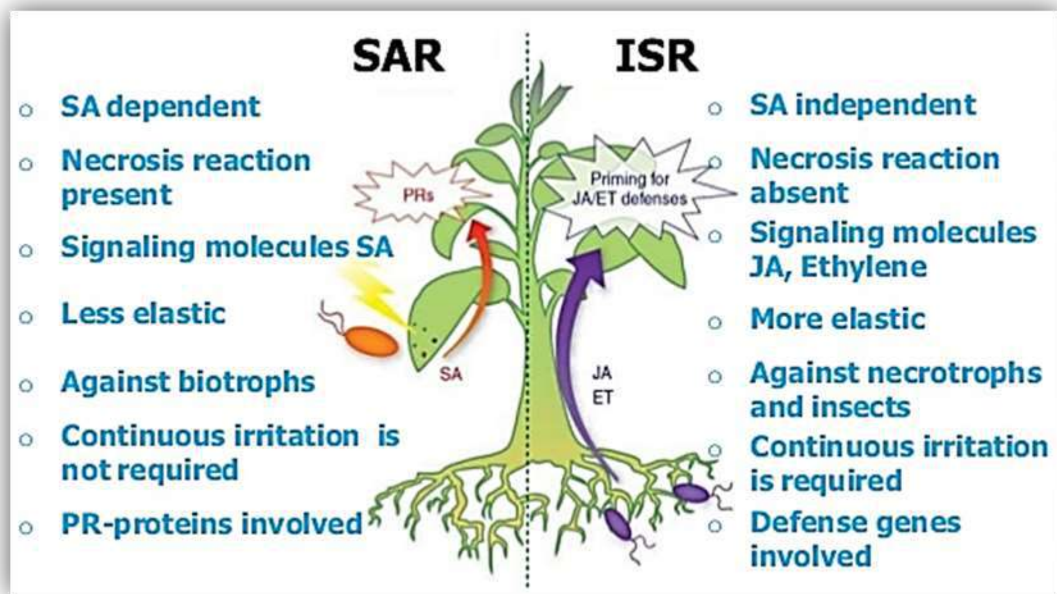
Hormones regulate a variety of plant behaviors in response to different stimuli or environmental conditions. The main hormones involved in plant defenses are:

❖ **Methyl Salicylate (MeSA) or Salicylic acid (SA)**

Methyl salicylate (MeSA) helps regulate responses to **infection** by parasites or pathogens. When a parasite or a pathogen infects a cell, there is a specific, localized response called the **hypersensitive response** (**HR**). Following this much localized response, the plant initiates a systemic (**whole body**) response called the **systemic acquired response** (**SAR**). **MeSA** is responsible for inducing the **SAR** in response to the **HR**. Usually; **SA** is required for fighting **biotrophic pathogens**.

❖ **Jasmonic acid (JA) or Jasmonate**

JA and its derivatives are plant signaling molecules closely related to plant defense and resistance to microbial pathogens, herbivorous insects, wounding, drought, salt stress, and low temperature. It plays a central role in plant defenses against **necrotrophic pathogens** and herbivorous insects. This pathway is also activated following the interaction with **beneficial microbes that** may lead to induced systemic resistance (**ISR**). ISR can induce **priming** effect which enables the plant to be ready for the second pathogen attack and respond in a much faster and stronger way than normal or basic response.



Systemic Acquired Resistance (SAR) and It's Significance in Plant Disease Management

LAB 9 ---------- The Plant defense mechanisms: Part 3 (Zigzag Model)

Terms you should understand and keep in mind before we start:

- **PAMPs** or **MAMPs** = **P**athogen or **M**icrobe **A**ssociated **M**olecular **P**attern are conserved molecules shared by many classes of microbes. Such as cell wall components, flagella, and Lipopolysaccharides LPS.

- **PRRs** = **P**attern **R**ecognition **R**eceptors that present on the plant side and perceive the PAMPs or MAMPs on the pathogen side. PRRs are usually receptor-like proteins or receptor-like kinases (RLPs or RLKs) that are attached to the cell membrane.

- **R-proteins are R**esistance proteins are intracellular receptors. Most of the identified R proteins belong to the **N**ucleotide **B**inding **L**eucine **R**ich **R**epeats (NB-LRR). They are mostly involved in the plant immunity when an effector from a pathogen is delivered into the plant cells.

- **PTI** = **P**AMP-**T**riggered **I**mmunity or now called **P**attern-**T**riggered **I**mmunity which is the first plant response to the pathogen attack.

- **ETI** = **E**ffector-**T**riggered **I**mmunity responds to virulence factors termed, effectors, that serve to suppress PTI. This part of defense is much stronger than PTI.

Plant pathogens use diverse life strategies. Pathogenic bacteria proliferate in intercellular spaces (the apoplast) after entering through gas or water pores (stomata and hydathodes, respectively), or gain access via wounds. Nematodes and aphids feed by inserting a stylet directly into a plant cell. Fungi can directly enter plant epidermal cells, or extend hyphae on top of, between, or through plant cells. These diverse pathogen classes all deliver effector molecules (virulence factors) into the plant cell to enhance microbial fitness.

- **Effector proteins** are proteins secreted by pathogens into the cells of their host, usually using type 3 secretion system (TTSS/T3SS), a type 4 secretion system (TFSS/T4SS) or a Type VI secretion system (T6SS). Some pathogens inject only a few effectors into their host's cells while others may inject dozens or even hundreds. Effector proteins may have many different activities, but usually help the pathogen to invade host tissue, suppress its immune system, or otherwise help the pathogen to survive.

- **ETS** = **E**ffector-**T**riggered **S**usceptibility is a term describes the ability of pathogens to suppress ETI via producing and injecting more virulence factors causing plant susceptibility and sensitivity.

In 2006, Jones and Dangl proposed a simple coevolutionary model of plant–pathogen interactions, called the ‘zigzag’ model, which encompasses two branches of the plant immune system. The first branch recognizes conserved molecules shared by many classes of microbes PAMPs and is called PTI. The second branch recognizes and responds to effectors that are able to suppress PTI. This branch of immunity is called ETI.

Based on **Molecular scope**, the zigzag model is based only upon interactions between the host immune system and biotrophic microbes that impair plant growth and reproduction.

The defined molecular participants in this model are:

1. On the plant's side, PRRs proteins

2. On the pathogen's side, MAMPs or PAMPs and ‘effectors that interfere with PTI or otherwise enable pathogen nutrition and spread’.

3. A severe ETI response from the plant is probably not a component in symbiosis (the plant ‘leaves the door open’). In necrotrophy, plant cell death might be seen as a favorable outcome for the microbe, thereby reversing the usual interpretation of induced cell death in ETI from ‘immunity’ to something more like ‘susceptibility’.

Ordering of events

The zigzag model describes four phases:

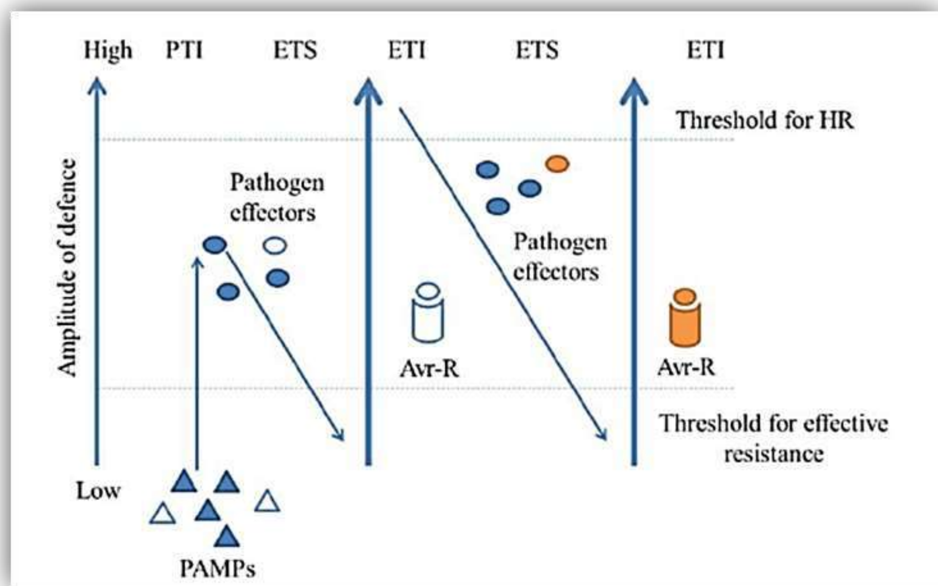
Phase 1: PAMPs are detected by PRRs leading to induce PTI

Phase 2: pathogens deliver effectors to interfere with PTI (ETS1)

Phase 3: effectors are recognized by R-proteins that will induce ETI

Phase 4: loss/gain of effectors over evolutionary time (ETS2).

Phase 4 occurs as a result of a selection pressure, which would act at a population



Zigzag model as described by Jones and Dangl

LAB 10 ----------- *How do phytochemicals work?*

Introduction

There are many phytochemicals and each works differently. These are some possible actions:

- **Antioxidant** - Most phytochemicals have antioxidant activity and protect our cells against oxidative damage and reduce the risk of developing certain types of cancer. Phytochemicals with antioxidant activity: allyl sulfides (onions, leeks, garlic), carotenoids (fruits, carrots), flavonoids (fruits, vegetables), polyphenols (tea, grapes).
- **Hormonal action** - Isoflavones, found in soy, imitate human estrogens and help to reduce menopausal symptoms and osteoporosis.
- **Stimulation of enzymes** - Indoles, which are found in cabbages, stimulate enzymes that make the estrogen less effective and could reduce the risk for breast cancer. Other phytochemicals, which interfere with enzymes, are protease inhibitors (soy and beans), terpenes (citrus fruits and cherries).
- **Interference with DNA replication** - Saponins found in beans interfere with the replication of cell DNA, thereby preventing the multiplication of cancer cells. Capsaicin, found in hot peppers, protects DNA from carcinogens.
- **Anti-bacterial effect** - The phytochemical allicin from garlic has anti-bacterial properties.
- **Physical action** - Some phytochemicals bind physically to cell walls thereby preventing the adhesion of pathogens to human cell walls. Proanthocyanidins are responsible for the anti-adhesion properties of cranberry. Consumption of cranberries will reduce the risk of urinary tract infections and will improve dental health.

How do we get enough phytochemicals?

- Foods containing phytochemicals are already part of our daily diet.

- In fact, most foods contain phytochemicals except for some refined foods such as sugar or alcohol.
- Some foods, such as whole grains, vegetables, beans, fruits and herbs, contain many phytochemicals.
- The easiest way to get more phytochemicals is to eat more fruit (blueberries, cranberries, cherries, apple,...) and vegetables (cauliflower, cabbage, carrots, broccoli,...).
- It is recommended take daily at least 5 to 9 servings of fruits or vegetable.
- Fruits and vegetables are also rich in minerals, vitamins and fiber and low in saturated fat.

Future of phytochemicals

- Phytochemicals are naturally present in many foods but it is expected that through bioengineering new plants will be developed, which will contain higher levels.
- This would make it easier to incorporate enough phytochemicals with our food.

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