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Ministry of Higher Education and
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College of Sciences
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Practical Labs of Plant Physiology

Second Stage

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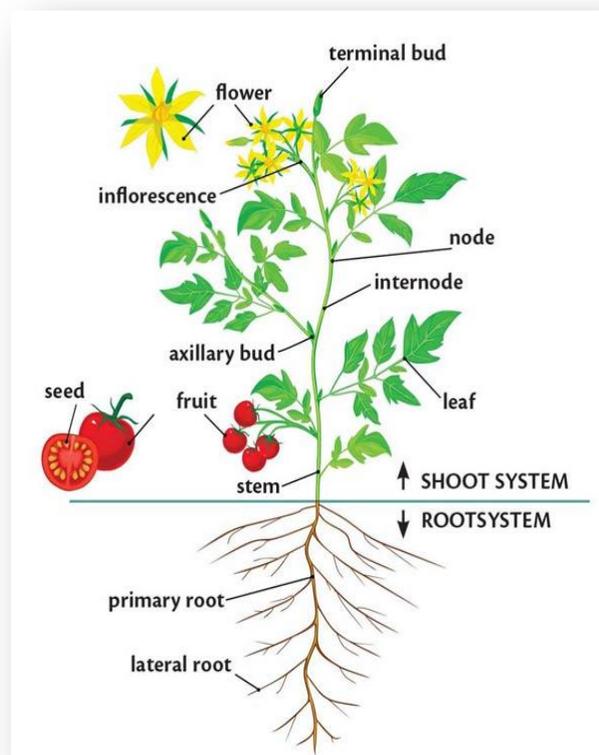
ORGANIZATIONS

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 plant have any) and fruit (if the plant have any).

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

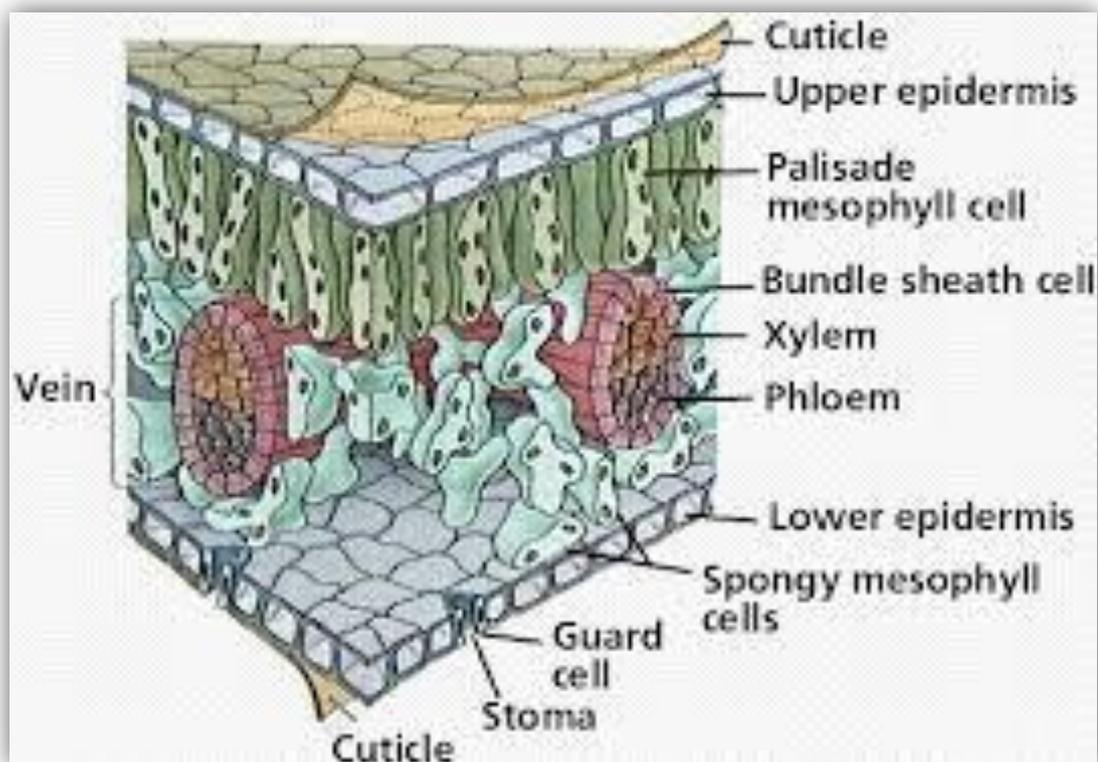


The whole plant

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.



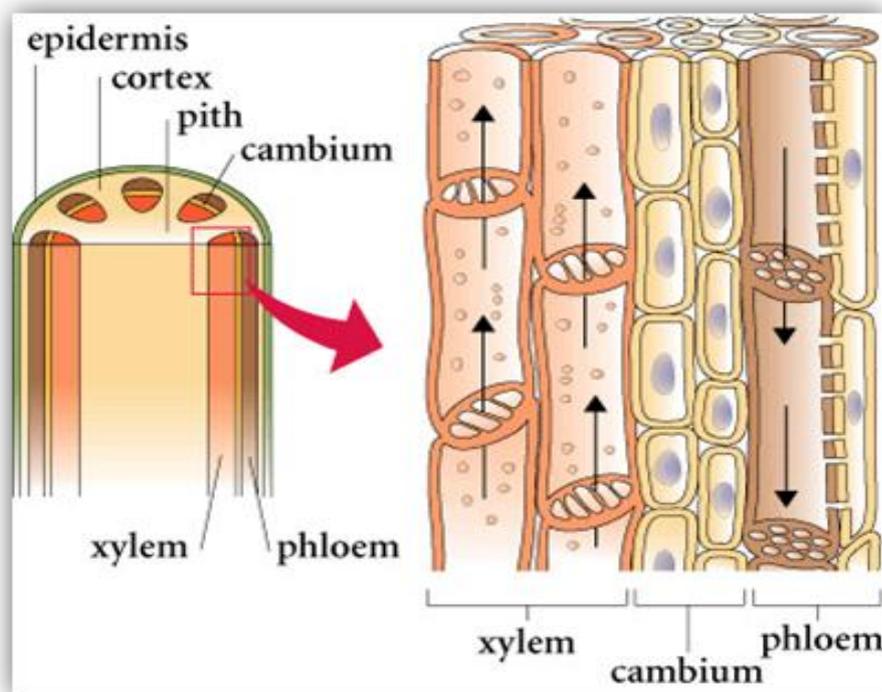
Plant Structure

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 Safranin-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.

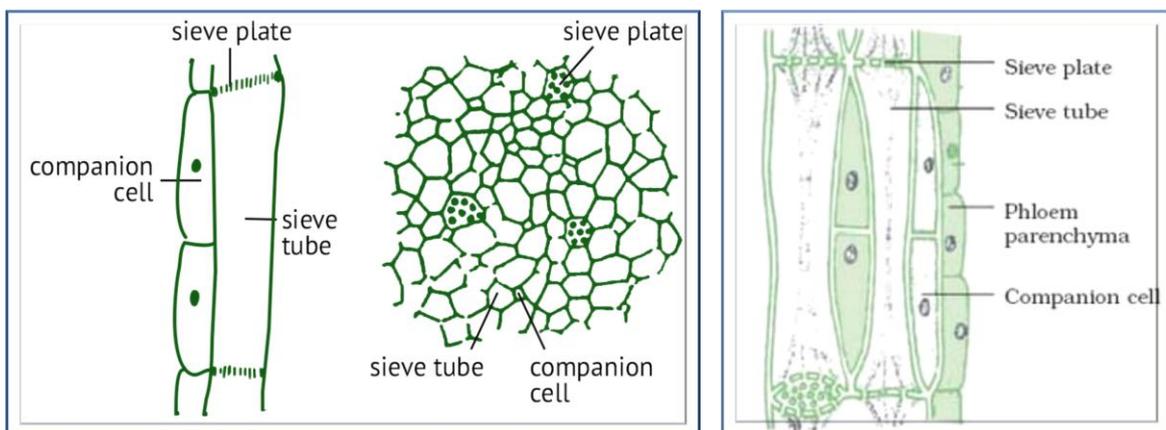


Xylem Structure

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 cells. Companion cells retain their nucleus and control the adjacent sieve cells. Dissolved food, as sucrose, flows through the sieve cells. Phloem cells as seen in longitudinal section.

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



Phloem Structure

The solutions

TERMS ABOUT SOLUTIONS:

- 1- **Solution**: Homogenous mixture.
- 2- **Solute**: The substance that is dissolved (small amount).
- 3- **Solvent**: The dissolving substance (large amount).
- 4- **Solvation**: The interactions between the solute and solvent.
- 5- **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 $< 1\text{nm}$).
- 2- Colloidal (diameter of particles $1\text{-}200\text{ nm}$).
- 3- Suspension (diameter of particles $> 200\text{ nm}$).

- **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 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.

The solutions

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).

Ex. Prepare 10% solution of sucrose. The solution is prepared by dissolving 10 gm of sucrose in a 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).

Ex. 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, its abbreviated with (M).

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

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).

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

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

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

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

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

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:

<u><i>Pigment Type</i></u>	<u><i>Colors</i></u>	<u><i>Found in</i></u>
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

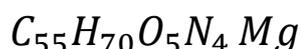
A) Chlorophylls:

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



2- **Chlorophyll b:** Greenish yellow in color with chemical formula



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.

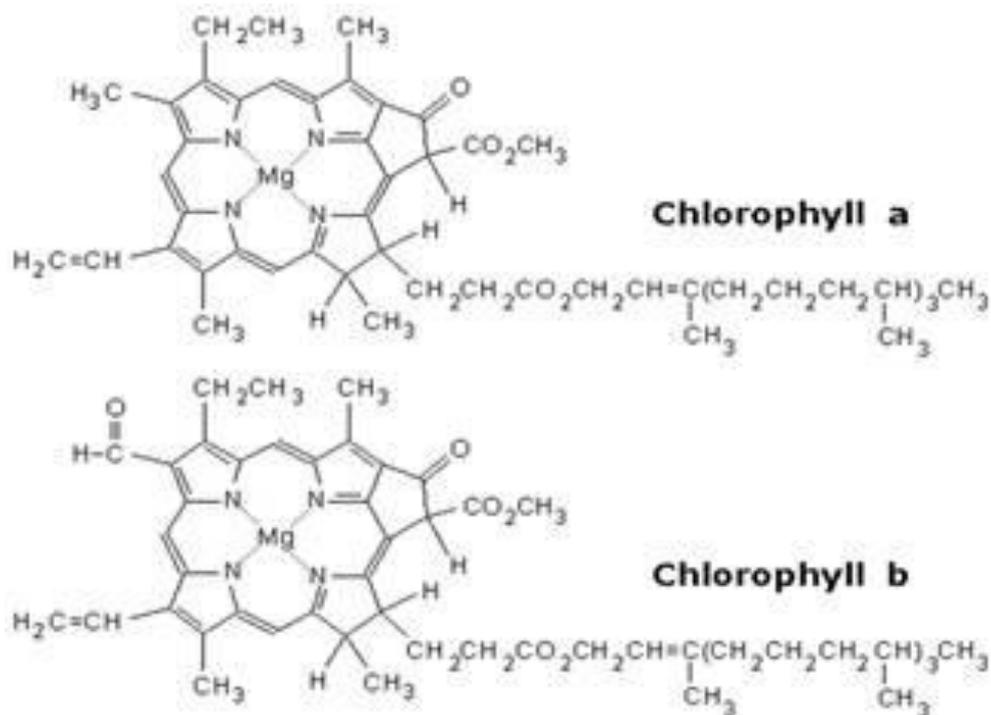
Plant pigments

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 $C_{55}H_{77}O_5N_4Mg$	Chemical formula $C_{55}H_{70}O_5N_4Mg$
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:

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

Increase the absorption spectrum of light.

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:

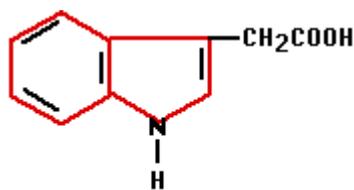
- a- Site of action.
- b- Development stage of plant.
- c- Concentration of hormone.

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)**.

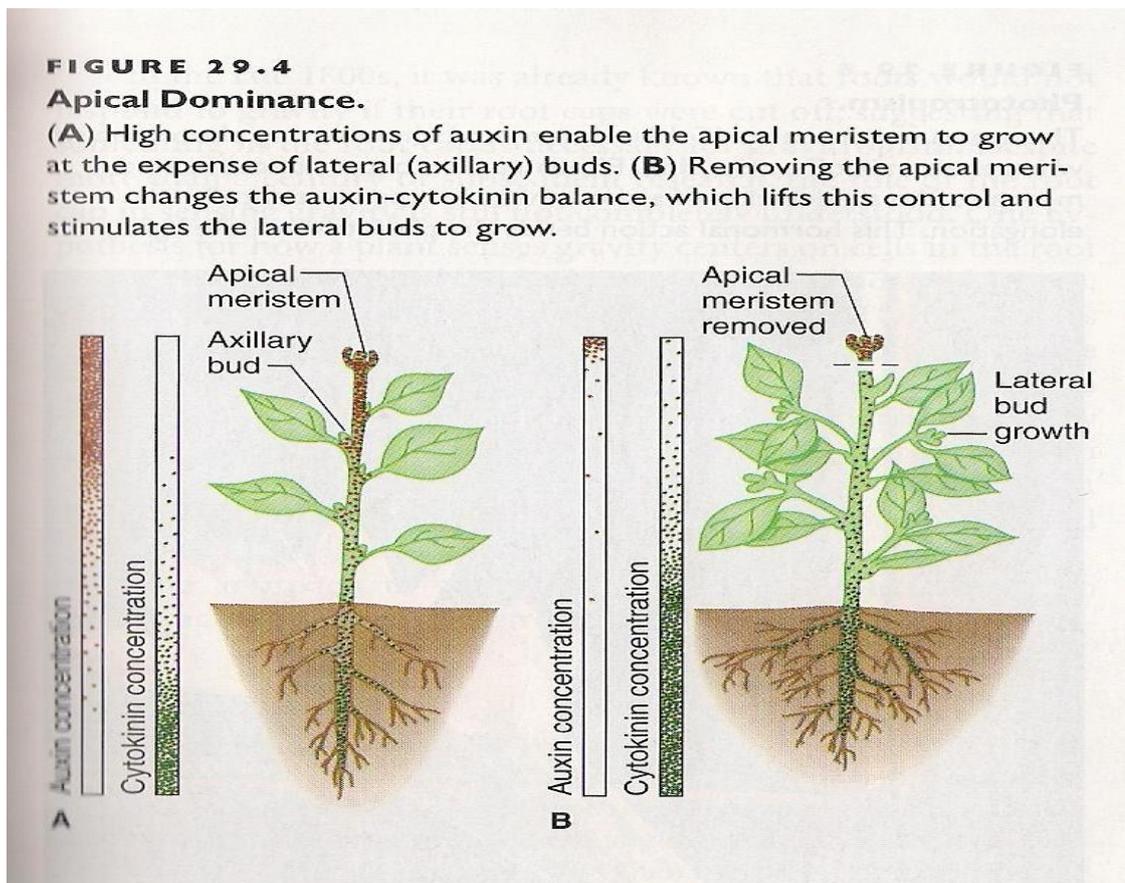


Indole-3-acetic acid (IAA)

**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.



The Plant Hormones

1- 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.

2- 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 α -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.

3- 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.

4- 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.

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 CO₂ and O₂ 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.

Leaf stomates are the primary sites of transpiration and consist of two guard cells that form a small pore on the surfaces of leaves. The guard cells control the opening and closing of the stomates in response to various environmental stimuli and can regulate the rate of transpiration to reduce water loss. Darkness and internal water deficit tend to close stomates and decrease transpiration; illumination, ample water supply, and optimum temperature open stomates and increase transpiration. Many plants close their stomates under high temperature conditions to reduce evaporation or under high concentrations of carbon dioxide gas, when the plant likely has sufficient quantities for photosynthesis.

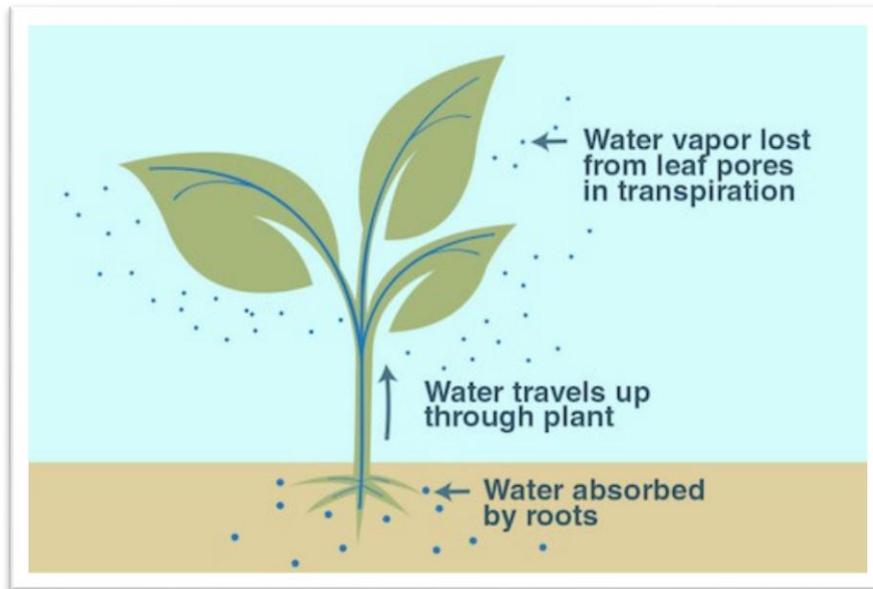


Fig.(1) Transpiration mechanism in plant body plantlet

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.

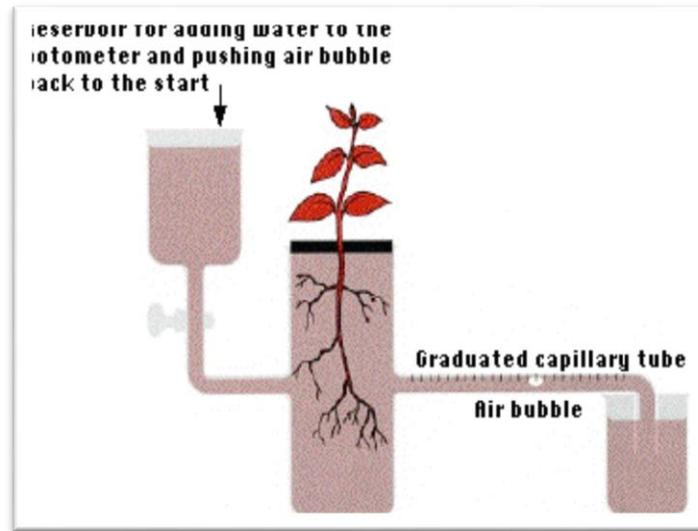


Fig.(1) The Potometer

Transpiration in plant

****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

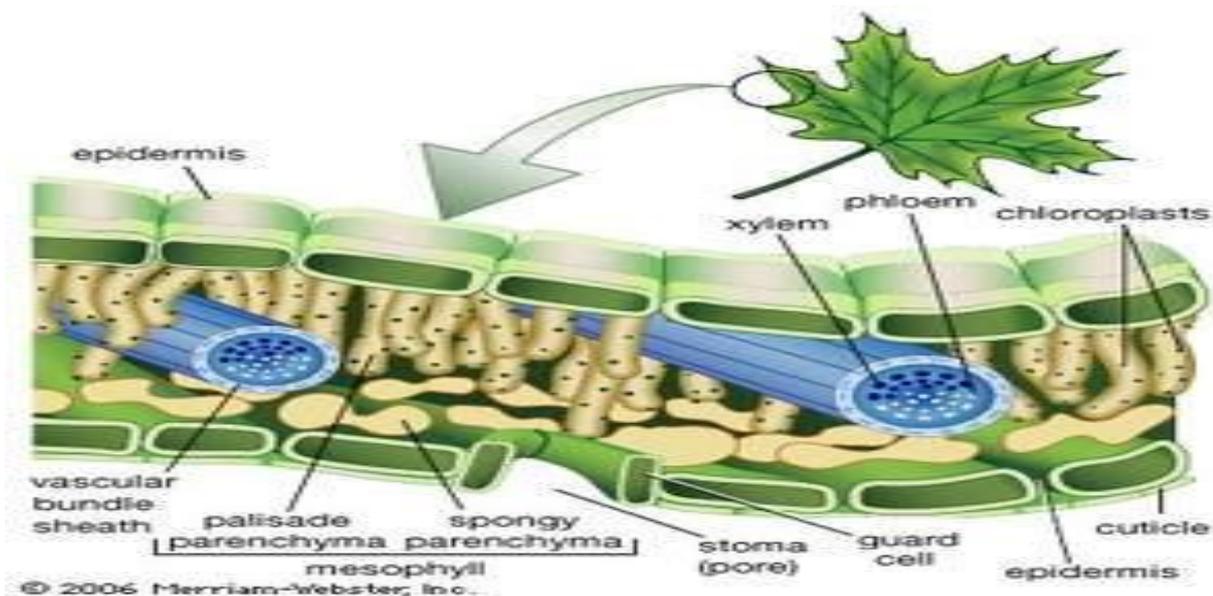
The rate of diffusion of any substance increases as the difference in concentration of the substances in the two regions increases. When the surrounding air is dry, diffusion of water out of the leaf goes on more rapidly.

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. 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.

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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