TRANSPORT IN PLANTS

Water relations

The Role of Solute Potential

What happens when the cell is transferred to the beaker containing pure water, which has no solutes. The solution surrounding the cell is strongly hypotonic relative to the cell-that is, the surrounding solution has a lower solute concentration than the cell. Although water moves across the membrane in both directions, under these conditions, there will be a net movement of water into the cell by osmosis. The tendency for water to move in response to differences in solute concentrations is determined by the solute potential.

Solute potentials are always negative because they are measured relative to the solute potential of pure water, and *solutions with high concentrations of solutes have low solute potentials*.
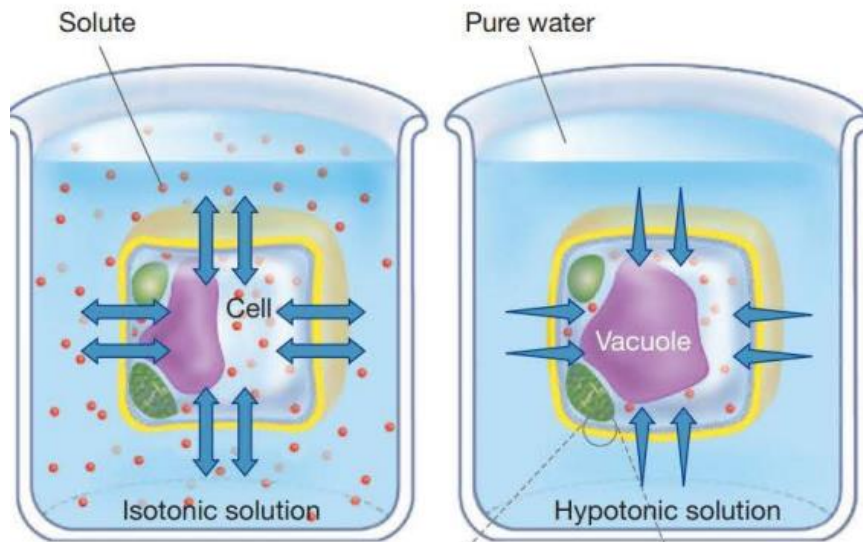
The Role of Pressure Potential

When an animal cell is placed in a hypotonic solution and water enters the cell via osmosis, the volume of the cell increases and the cell may even burst. This does not happen to plant cells, however. If a plant cell swells in response to incoming water, its plasma membrane pushes against the relatively rigid cell wall. The cell wall resists expansion of the cell volume by pushing back, just like a bicycle tyre pushes back against its inner tube as it is inflated. The force exerted by the wall is called wall pressure. As water moves into the cell, the pressure of the fluid contents inside the cell, known as turgor pressure, increases until wall pressure is induced. Cells that are firm and at experience wall pressure are said to be turgid.

Note: Wall pressure and turgor pressure are equal but opposite forces

When solute potential and pressure potential affect a cell at the same time, the combined effect of these two forces determines the net direction of water flow.
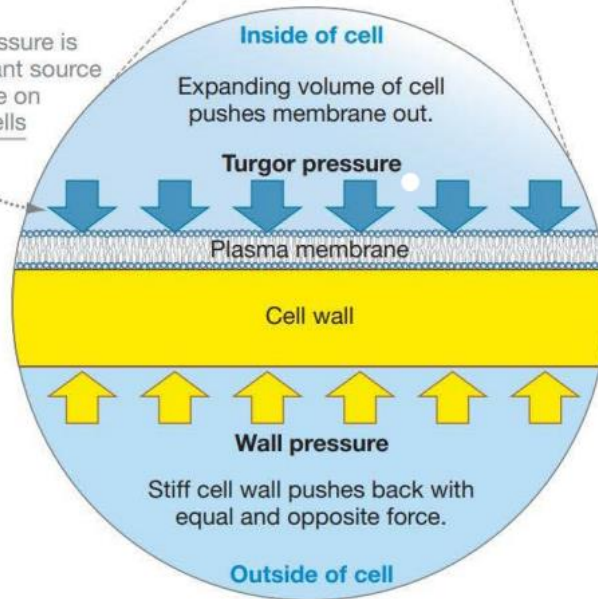
2

a) Solute potential is the tendency of water to move by osmosis.

- *Solute potential inside cell and in surrounding solution is the same. No net movement.*
- *Cell is placed in pure water. The cell's solute potential is low relative to its surroundings. There's net movement of water into cells.*

Solute

Pure water

Cell

Vacuole

Isotonic solution

Hypotonic solution

(b) Pressure potential is the tendency of water to move in response to pressure.

Turgor pressure is an important source of pressure on water in cells

Inside of cell

Expanding volume of cell pushes membrane out.

Turgor pressure

Plasma membrane

Cell wall

Wall pressure

Stiff cell wall pushes back with equal and opposite force.

Outside of cell

Working with Water Potentials

If you ignore the effects of gravity, which are negligible over short distances such as between cells, water potential is defined by the following equation: $\Psi w = \Psi s + \Psi p$

In other words, the potential energy of water in particular location is the sum of the solute potential and pressure potential existing at that site.

Although solute potentials are always negative, the pressure potential from turgor pressure is positive inside cells, though in dead cells like xylem, pressure potential can sometimes be negative.

Water movement in the absence of pressure.

In the U – shaped tube on the left of the figure (a), two solutions are separated by a semi permeable membrane. The system is open to the atmosphere and thus not under any additional pressure, meaning, $\Psi p = 0MPa$.

Note that the left side of the tube contains pure water, which has a Ψw of 0MPa. The Ψs of the solution on the right is -0.1MPa. Because water potential is higher on the left than on the right side of the tube, the overall net movement of water is from left to right.

The right side of figure (a) illustrates the same situation with a cell that is initially flaccid, meaning it has no turgor pressure and therefore has a pressure potential of 0MPa. Note that the cell has been placed in a solution of pure water. Because the cell has a low solute potential (-0.1MPa) and the pure water has a higher water potential than the cell, water enters the cell by osmosis.
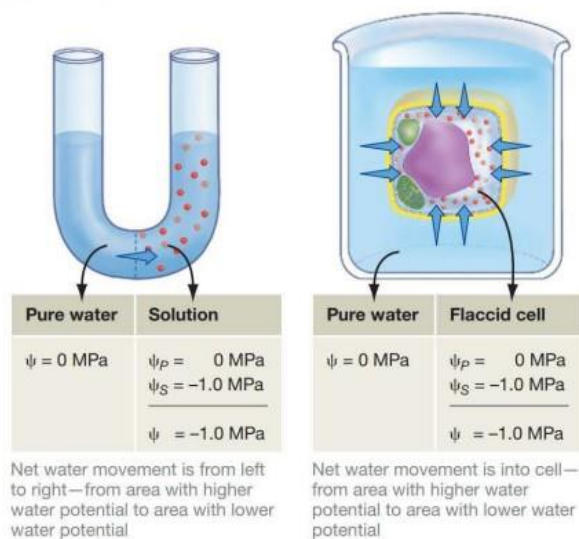
Water movement in the presence of solute and pressure potentials

On the left side of figure (b), the concentrations in the U-shaped tube are the same as in figure (a), but the solution on the right side of the tube experiences pressure exerted by a plunger/piston. If the force on the plunger produces a pressure potential of 1.0MPa on the right side, and if the Ψs for the solution on the right side of the membrane is still -0.1MPa, then the water potential of the right side is -0.1MPa + 1.0MPa = 0.
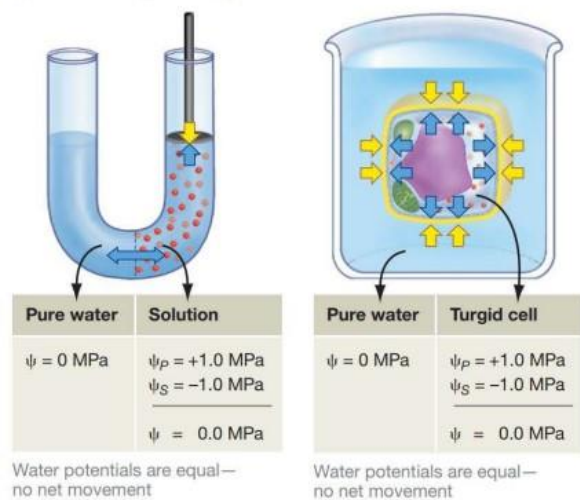
In this case, the water potential on both sides of the membrane is equal and there will be no net movement of water. If the force on the plunger is greater than 1.0MPa, the solution on the right side will have a higher water potential and water will flow from the right to the left.

The right side of the figure (b) models this situation in a living cell, where the incoming water creates turgor pressure. When the positive turgor pressure (+0.1MPa) plus the cell's negative solute potential (-0.1MPa) equals 0MPa, the system reaches equilibrium. At equilibrium, there is no additional net movement of water. In this way, turgor pressure acts like the plunger in figure (b).

(a) Solute potentials differ.

(b) Solute and pressure potentials differ.

Pure water	Solution
$\psi = 0$ MPa	$\psi_P = \quad 0$ MPa
	$\psi_S = -1.0$ MPa
	$\psi = -1.0$ MPa

Net water movement is from left to right—from area with higher water potential to area with lower water potential

Pure water	Flaccid cell
$\psi = 0$ MPa	$\psi_P = \quad 0$ MPa
	$\psi_S = -1.0$ MPa
	$\psi = -1.0$ MPa

Net water movement is into cell—from area with higher water potential to area with lower water potential

Pure water	Solution
$\psi = 0$ MPa	$\psi_P = +1.0$ MPa
	$\psi_S = -1.0$ MPa
	$\psi = \quad 0.0$ MPa

Water potentials are equal—no net movement

Pure water	Turgid cell
$\psi = 0$ MPa	$\psi_P = +1.0$ MPa
	$\psi_S = -1.0$ MPa
	$\psi = \quad 0.0$ MPa

Water potentials are equal—no net movement

Water Potentials in Soils, Plants, and the Atmosphere

The water contained in leaf, stem, or root tissues has a pressure potential and a solute potential, just like the water inside a cell does. Likewise, both the soil surrounding the root system and the air around the shoot system have a water potential.

Water Potential in Soils: In moist soil, the water that fills crevices between soil particles usually contains relatively few solutes and normally is under little pressure. As a result, its water potential tends to be high relative to the water potential found in a plant's roots, which is higher in solutes. There are important exceptions to this rule, however:

• Sal soils: Soils near ocean coastlines have water potentials as low as -4 MPa or less due to high solute concentrations. This value is much lower than the water potential typically found inside plant roots.

• Dry soils: When soils are dry, water no longer flows freely in the spaces between soil particles. All of the remaining water adheres tightly to soil particles, creating a tension that lowers the water potential of soil water. When the water potential in soil drops, water is less likely to move from soil into roots. If soil water potential is low enough

How Are Some Plants Specialized for Salty or Dry Soils?

Salt adapted species often respond to low water potentials in soil by accumulating solutes in their root cells, which then lowers their solute potential. These plants have enzymes that increase the concentrations of certain organic molecules in the cytoplasm. Thus, they can keep the water potential of their tissues lower than that of salty soils and are able to absorb what little water is available. Species that are adapted to dry soils cope by tolerating low solute potentials.

Water Potential in Air

 In the atmosphere, water exists as a vapor with no solute potential. The pressure exerted by water vapor in the atmosphere depends on temperature and humidity. The lower the pressure potential, the faster liquid water evaporates into the atmosphere.

• When air is dry, few water molecules are present and the pressure they exert is low, increasing the rate of evaporation.

• When air is warm, water molecules move farther apart and also exert lower pressure.

Warm, dry air has an extremely low water potential, often approaching -100 MPa. When the weather is cool and rainy or foggy, however, the water potential of the atmosphere may be equal to the water potential inside a leaf. But normally, the water potential of the atmosphere is lower than the water potential inside a leaf, so water in the leaf evaporates quickly.

In most cases, water potential is highest in soil, medium to high in roots, low in leaves, and very low in the atmosphere. This situation sets up a water-potential gradient that causes water to move up through the plant. To move up a plant, water moves down the water potential gradient that exists between the soil, its tissues, and the atmosphere. When it does so, it replaces the water lost through transpiration

Questions:

1. Explain how grass roots can lose water instead of absorb water when a lawn is overfertilized.
2. Explain whether the roots of pickleweed (Salicornia europaea), a plant that grows well in salty soil, are likely to have a higher or lower water potential than the surrounding soil.

MOVEMENT OF WATER AND SOLUTES INTO THE ROOT

To understand how water enters a root, consider the cross section through a young buttercup root shown in the figure below. Starting at the outside of the root and working inward, notice that several distinct tissues are present:

• The **epidermis** (literally, "outside skin") is a single layer of cells. In addition to protecting the root, some epidermal cells produce long, thin root hairs, which greatly increase the total surface area of the root.

• The **cortex** consists of ground tissue and stores carbohydrates.

• The **endodermis** ("inside skin") is a cylindrical layer of cells that forms a boundary between the cortex and the vascular tissue. The function of the endodermis is to control ion uptake and prevent ion leakage from the vascular tissue.

• The **pericycle** ("around circle") is a layer of cells located just interior to the endodermis that forms the outer boundary of the vascular tissue. The pericycle can produce lateral roots.

• Conducting cells of the vascular tissue, **xylem** and **phloem**, transport water and nutrients between roots and shoots and are located in the centre of roots in buttercups and other eudicots. Notice that, in these plants, phloem is situated between each of four arms formed by xylem, which is arranged in a cross-shaped pattern.
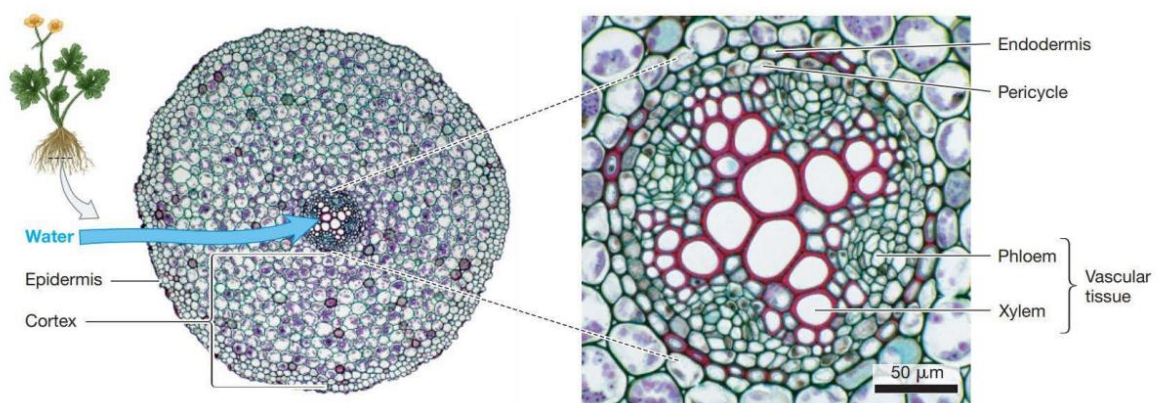
Root hairs as exchange surfaces

Root hairs are an example of specialised exchange surface in plants. They are responsible for absorption of water and mineral salts. Plants constantly lose water by the process of transpiration, and this loss can be up to 700dm^3 a day in a large tree, all of which must be replaced by water that is absorbed through the root hairs.

Each root hair is a tiny extension of a root epidermal cell. These root hairs remain functional for a few weeks before dying back to be replaced by others close to the growing root tip. *They are efficient surfaces for the exchange of water and mineral ions because*:

- They provide a large surface area because they are very long extensions and occur in their thousands on each of the branches of a root.
- They have a thin cell surface membrane, reducing the exchange distance across which materials can move.
- They are permeable; the epidermal cell is not covered by a waxy cuticle and the thin cellulose cell wall is no barrier to the movement of water and ions.
- The cell surface membrane has specialised protein channels called aquaporins to allow water to pass across it more easily
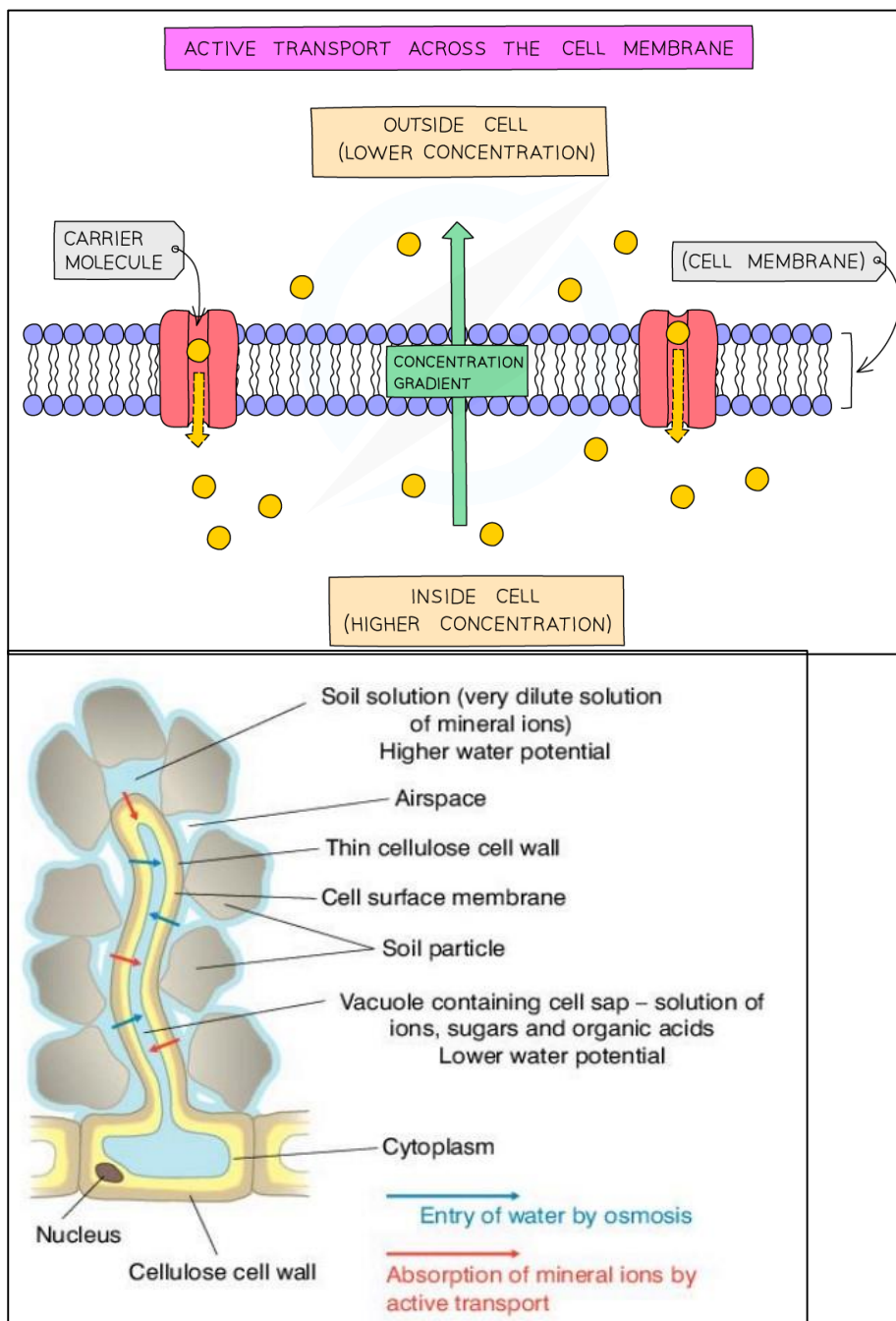
Absorption of water.

Water enters root hair cells by a passive process of osmosis. The soil solution has a very low concentration of mineral ions in water and surrounds the particles that make up soil. The solution therefore has a high water potential. Root hair cells, in comparison, have a relatively high concentration of ions, sugars and organic acids within their cell vacuoles and cytoplasm, making the cells to have a lower water potential. Because the roots are in direct contact with the soil solution, water moves by osmosis from the higher (less negative) water potential of the soil solution to the lower (more negative) water potential within the root hair cells. From the root hair cells, water moves further into the root due to the water potential gradient that exists between it and the xylem in the centre of the root.
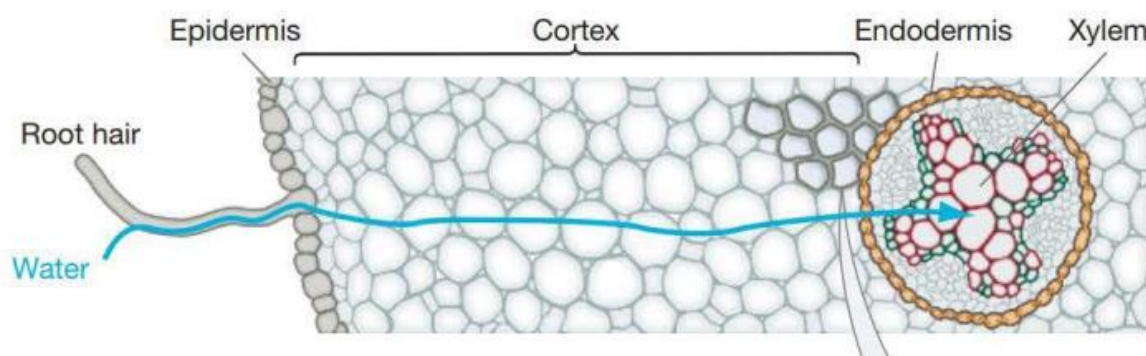
Mineral salts absorption.

Conversely, the absorption of mineral ions by the root hairs is through a completely different mechanism. The concentration of ions inside the root hair cell is normally greater than that in the soil solution. The uptake of mineral ions is therefore against the concentration gradient and, as a result, requires active transport. This is achieved using special carrier proteins that use ATP to provide energy to transport particular ions from the soil solution, where they are in lower concentrations, to the root hair cytoplasm and vacuole where they are at higher concentrations. On rare occasions when a particular ion is in a greater concentration in the soil than in the root hair cell, the ion simply moves into the cell by the passive process of facilitated diffusion.

ACTIVE TRANSPORT ACROSS THE CELL MEMBRANE

OUTSIDE CELL
(LOWER CONCENTRATION)

CARRIER MOLECULE

(CELL MEMBRANE)

CONCENTRATION GRADIENT

INSIDE CELL
(HIGHER CONCENTRATION)

Soil solution (very dilute solution of mineral ions)
Higher water potential

Airspace

Thin cellulose cell wall

Cell surface membrane

Soil particle

Vacuole containing cell sap – solution of ions, sugars and organic acids
Lower water potential

Cytoplasm

Nucleus

Cellulose cell wall

Entry of water by osmosis

Absorption of mineral ions by active transport

Three Routes through Root Cortex to Xylem

When water enters a root along a water potential gradient, it does so through root hairs. As water is absorbed, it moves through the root cortex toward the xylem along three routes, namely:

- The symplast pathway
- Vacuolar pathway
- The apoplast pathway

The symplast pathway.

This takes place across the cytoplasm of the cortical (cortex) cells as a result of difference in the water potential between cells. Water moves from one cell to the next cell through plasmodesmata, down the water potential gradient. Plasmodesmata are strands of cytoplasm that directly connect one cell to the adjacent cell, allowing water to take a cytoplasmic route across the cortex. There is therefore, in effect, a continuous column of cytoplasm extending from the root hair cell to the xylem at the centre of the root. Water moves along this column as follows:

- Water entering the root hair cell by osmosis raises its water potential.
- The root hair cell gains a higher water potential than the adjacent cortical cell.
- Water therefore moves from the root hair cell to the adjacent cortical cell via plasmodesmata, down the water potential gradient.
- This first cortical cell now has a higher water potential than the adjacent cell nearer to the centre of the root.
- Water therefore moves into this neighbouring cortical cell via plasmodesmata along the water potential gradient.
- This second cortical cell now has a higher water potential than the next cell nearer to the centre of the root, and so water moves from the second to the third cell via plasmodesmata down the water gradient.
- At the same time, this loss of water from the firsts cortical cell lowers its water potential, causing more water to enter it from the root hair cell.
- In this way, a water potential gradient is set up across all cells of the cortex, which carries water along the cytoplasm from the root hair to the endodermis, surrounding the central xylem.

Vacuolar pathway.

The vacuolar pathway of water movement in roots involves water passing directly through the vacuoles of plant cells. It is a slower process compared to the apoplast and symplast pathways. In this pathway, water moves from one vacuole to another through the tonoplast (the vacuolar membrane), crossing the cytoplasm and plasmodesmata between cells. This pathway allows for tighter regulation of water flow as it moves through the cells' internal structures.

Apoplast pathway

The apoplast pathway involves the movement of water through the cell walls and intercellular spaces without crossing the cell membrane. Water moves freely through the apoplast due to cohesion and adhesion forces, bypassing the living parts of the cell. As water is drawn into endodermal cells, it pulls more water long behind it, due to the cohesive properties of the water molecules. This creates a tension that draws water along the cell walls of the cells of the root cortex (cortical cells). The mesh-like structure of the cellulose cell walls of the cortical cells has many water-filled spaces and so there is little or resistance to this pull of water along the cell walls.

The apoplast pathway can be described as a non-living pathway as water does not enter the cytoplasm of cells. It is a faster route since it avoids the need to pass through the cell's cytoplasm. However, at the endodermis, water must eventually enter the symplast pathway due to the Casparian strip, which blocks further apoplastic flow.
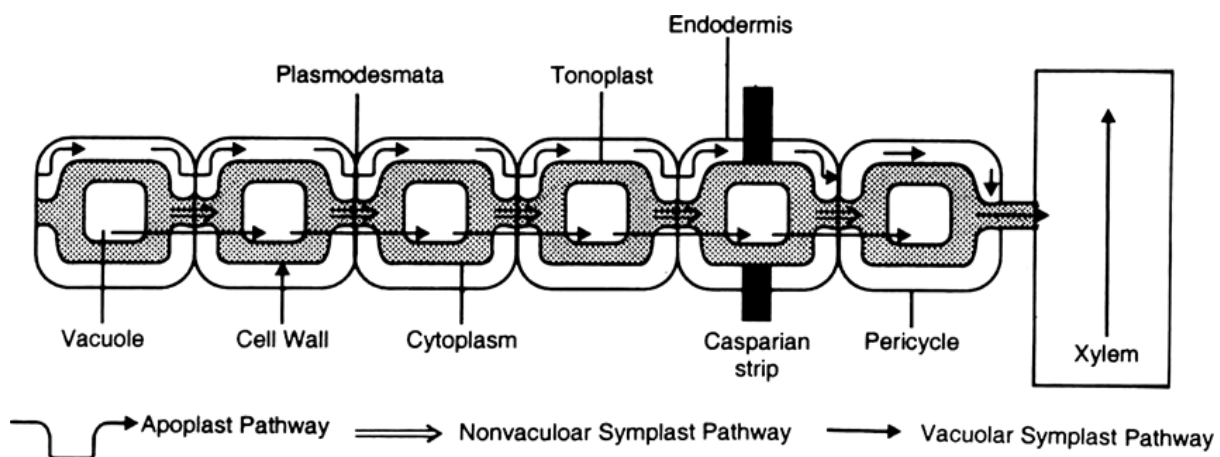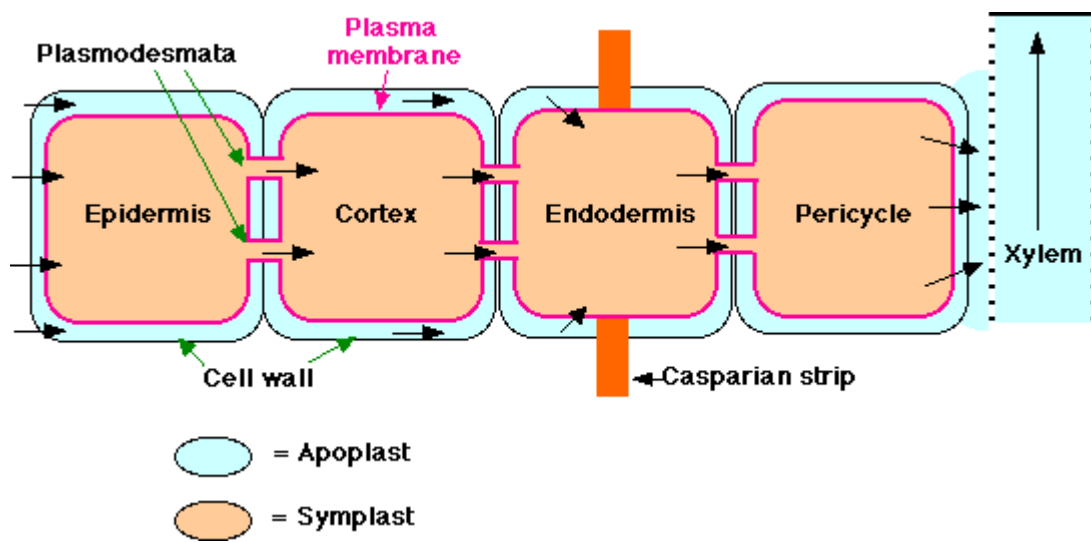
The Role of the Casparian Strip

The situation changes when water reaches the endodermis. Endodermal cells are tightly packed and secrete a narrow band of wax called the Casparian strip. This layer is composed primarily of

of the leaf, increasing in diameter towards the petiole, or leaf stalk. Within each vein, or vascular bundle, there is an area off xylem towards the upper surface of the leaf and an area of the phloem towards the lower surface. This arrangement is shown in the section through the leaf shown in the figure (a) below.
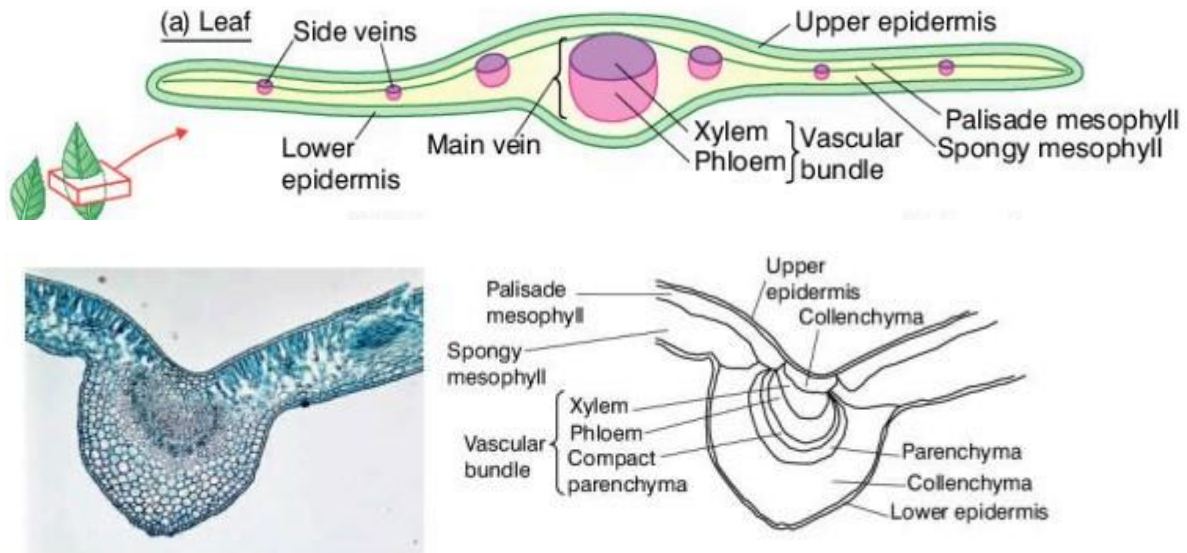
Figure Low power plan diagram (right) of the tissues shown in the photograph (left) of a TS through the midrib of a dicotyledonous leaf

Distribution of vascular tissue in a stem

The xylem and phloem in a dicotyledonous stem form vascular bundles that are arranged toward the outside of the stem. The reason for this is that the vascular bundles, together with associated sclerenchyma fibres, provide support in herbaceous stems as well as transporting materials.

The main forces acting on stems are lateral ones causes by the action of wind on them. These are best resisted by an outer cylinder of supporting tissue. Hence the vascular bundles form a non-continuous ring towards the edge of the stem. Being non-continuous, this ring of supporting tissues allows the stem to be flexible and to bend in the wind. Within the vascular bundles, the xylem is to the inside of the stem and the phloem is to the outside. Between the two is a thin layer of diving cells called the **cambium**, which gives rise to both xylem and phloem.
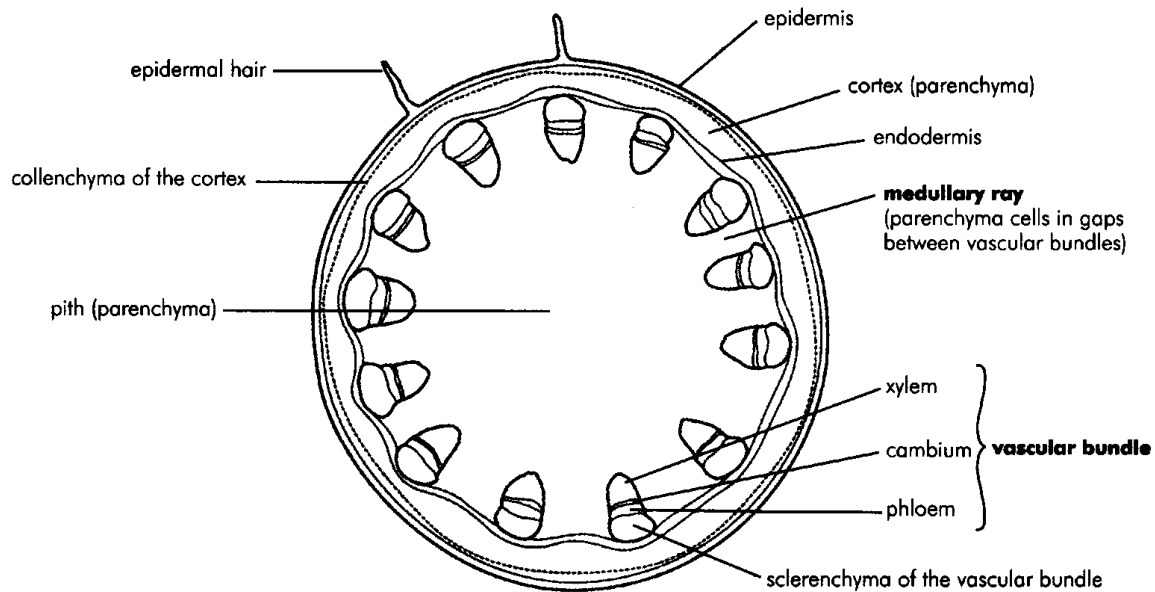
Vascular tissues in a stem

Distribution of vascular bundles in a root.

The vascular tissue in the root of a dicotyledonous plant is situated centrally rather than towards the outer edge, as in a stem. This is because roots are subject only to pulling forces. Vertical forces are better resisted by a central column of supporting tissues, such as xylem, rather than an outer cylinder of tissue. The xylem is typically arranged in a single star – shaped block of tissue at the centre of the root, with the phloem situated in separate groups between each of the points of the star-shaped xylem. Around both is the pericycle and endodermis.
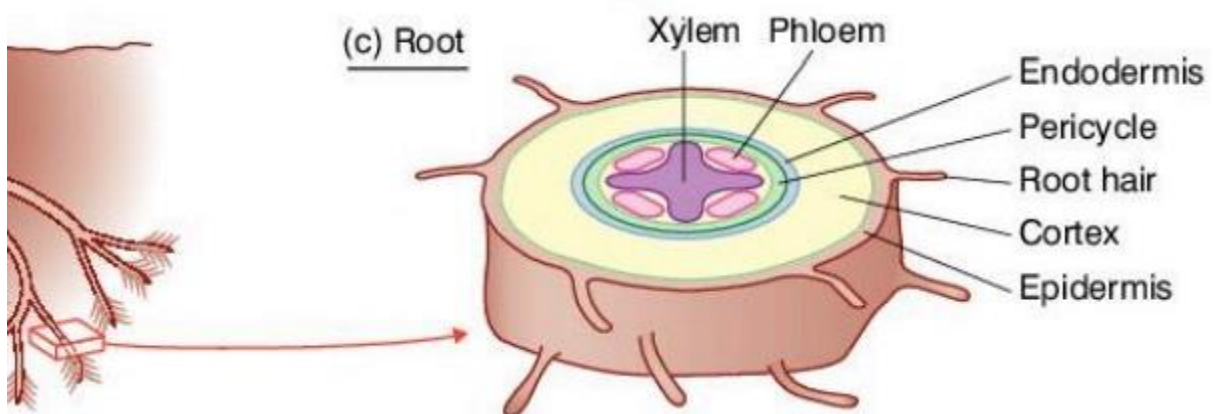
Monocot stem and root transverse sections

Anatomy of Monocot Root (*Colocasia*): Diagrammatic

Monocot Stem

Phloem
Xylem
Ground tissue
Vascular Bundle

Bundle sheath
Metaxylem
Protoxylem

Dicot Stem

Metaxylem
Protoxylem

Collenchyma of the cortex
Epidermal Hair
Epidermis
Edodermis
Bundle cup fibers
Phloem
Cambium
Xylem
Cortex
Pith
Medullary ray
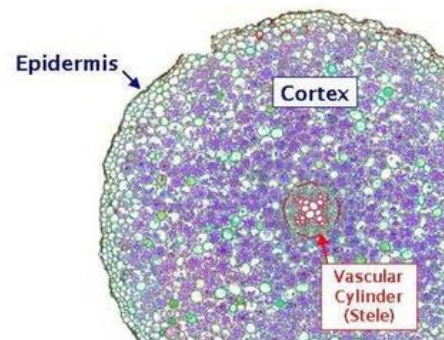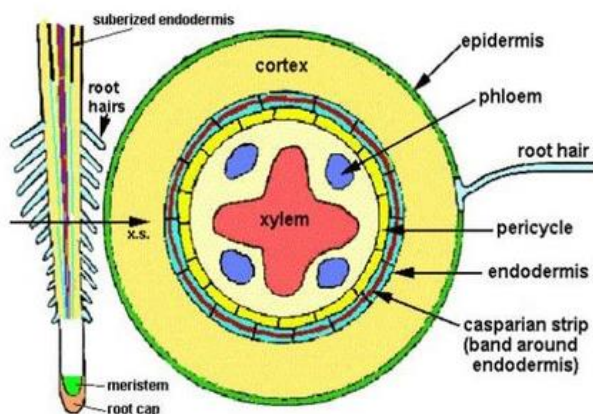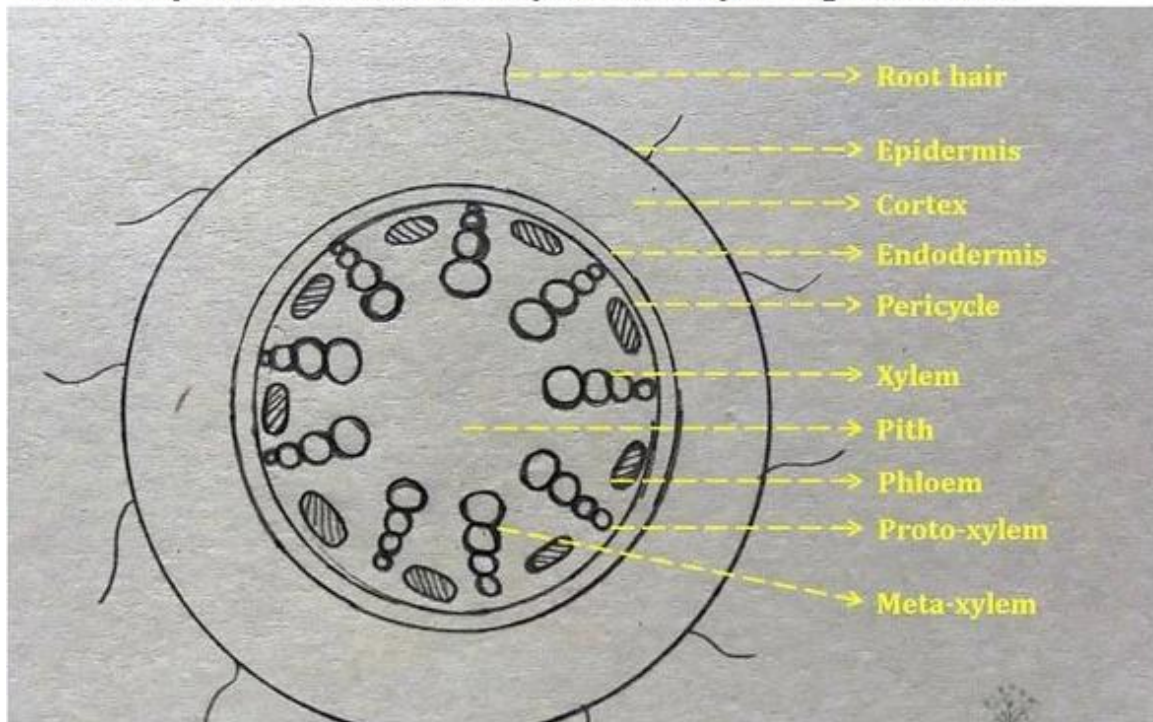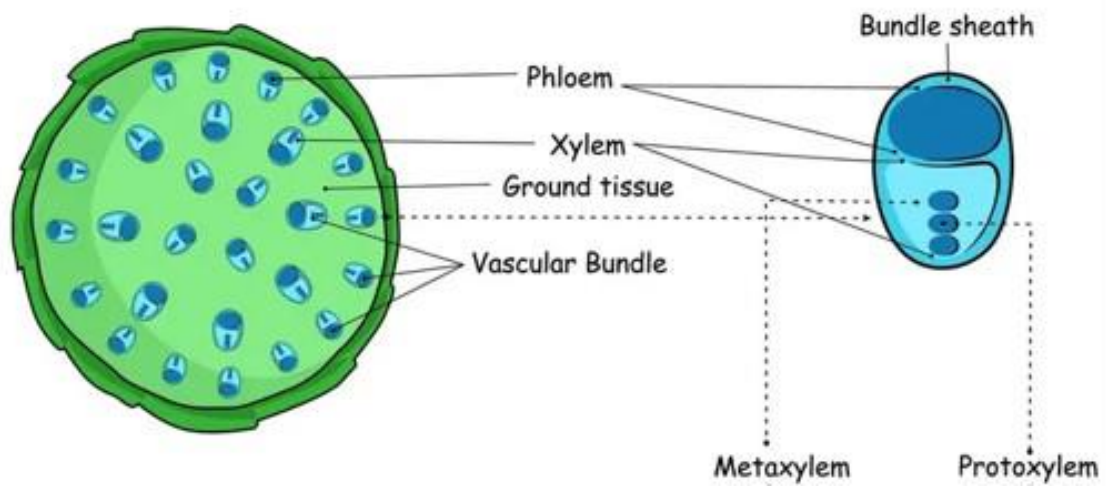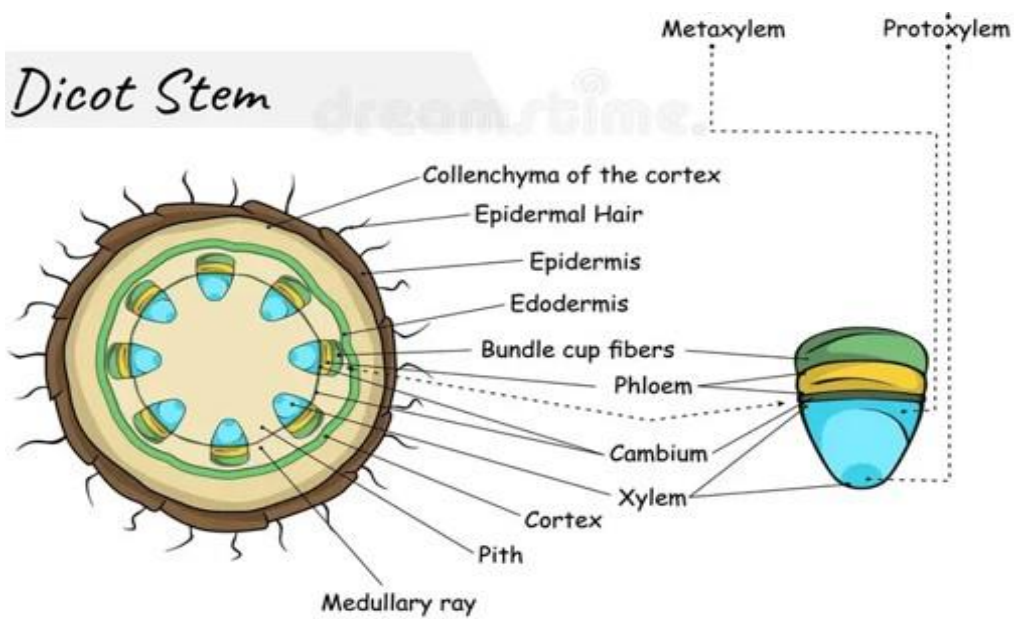
WATER ACCENT UP THE PLANT AND ACROSS THE LEAF.

The main force that pulls water up the stem of a plant is the evaporation of water from surfaces of the spongy mesophyll cells of leaves into the air spaces, from where the water vapour diffuses out of the atmosphere through open stomata. The loss of water from the aerial parts of a plant (mostly the leaves) is termed as transpiration. It is therefore logical to begin this topic from the point where water molecules evaporate from the spongy mesophyll cells and diffuse through the stomata into the atmosphere.

<u>Movement across the leaf</u>

The humidity of the atmosphere is usually less than that of the sub-stomatal air space (air space inside the leaf, just below the stomata) and so, provided that the stomata are open, water diffuses out of the air spaces into the surrounding air, which has a lower water potential.

Water vapour lost from the air spaces is replaced by water vapour evaporating from the cell walls of the surrounding spongy mesophyll cells. By closing stomatal pores, plants can control water loss. Water vapour evaporating from the spongy mesophyll cells is replaced by the adjacent spongy cells across a water potential gradient, and subsequently by the water reaching them from the xylem. Water movement occurs because once the spongy mesophyll cells have lost water to the sub-stomatal space, they have a lower water potential, causing influx of water from adjacent cells.

<u>Movement of water up the stem</u>

Suppose that you are caring for the wilted plant. If you add water to the soil, the water potential of the soil will increase and water will move into the roots down a water potential gradient. Water moves into root cells by osmosis, but how does it move up to the shoots, against gravity? Biologists have tested three major hypotheses for how water could be transported to shoots:

1. Root pressure-a pressure potential that develops in roots could drive water up against the force of gravity.
2. Capillary action could draw water up the cells of xylem.
3. Cohesion-tension, a force generated in leaves by transpiration, could pull water up from roots.

<u>Movement of water via root pressure.</u>

Movement of ions and water into the root xylem is responsible for the process known as root pressure. Recall that root pressure is one of three hypothesized mechanisms for moving water up xylem, from root to shoot. The Casparian strip in endodermal cells is essential for root pressure to develop. Without an apoplastic barrier between the xylem and the environment, ions and water would simply leak out of roots.

Stomata normally close during the night, when photosynthesis is not occurring and CO_2 is not needed. Their closure minimizes water loss and slows the movement of water through plants. "But

roots often continue to accumulate ions at their epidermal cells acquired from the soil as nutrients, and these nutrients are actively pumped into the xylem. The influx of ions lowers the water potential of root xylem below the water potential in the surrounding cells. As a result, water from nearby cells enters xylem. As water flows into xylem from other root cells in response to the solute gradient, a positive hydrostatic pressure is generated at night that forces fluid up the xylem."

That explains why more water moves up xylem and into leaves than is being transpired from the leaves. In certain low-growing plants, such as strawberries, enough water can move to force water droplets out of the leaves by a phenomenon known as **guttation**. If you have ever walked barefoot through the grass early in the morning, some of the water at gets your feet wet spent part of the night moving through plants.

At one time, positive root pressure formed the basis of a leading hypothesis to explain how water moves from roots to leaves in trees. However, follow-up research showed that over long distances, such as *from the ground to the top of a large tree, the force of root pressure is not enough to overcome the force of gravity on the water inside xylem*. In addition, researchers demonstrated that cut stems, which have no contact with the root system, are still able to transport water to leaves. Biologists concluded that there must be some other mechanism involved in the long-distance transport of water.

Figure 35.8 Root Pressure Causes Guttation. When ions accumulate in the xylem of roots at night, enough water enters the xylem via osmosis to force water up and out of low-growing leaves.
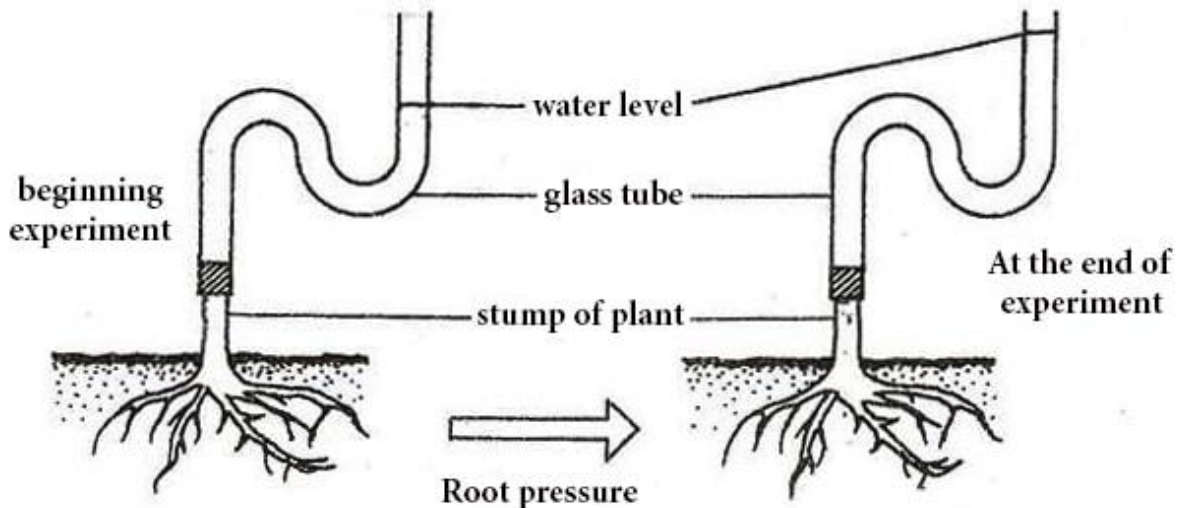
Water Movement via Capillary Action

Researchers have also evaluated a hypothesis based on the phenomenon of capillary action, or movement of water up a narrow tube.

When a thin glass tube (similar to xylem cells) is placed upright in a pan of water, water creeps up the tube. The movement occurs in response to three forces: (1) surface tension, (2) adhesion, and (3) cohesion. Let's briefly consider each force in turn.

Surface tension is a force that exists among water molecules at an air-water interface. In the body of a water column, all the water molecules are surrounded by other water molecules and form hydrogen bonds in all directions. Water molecules at the surface, however, can form hydrogen bonds only with the water molecules beside and below them. Because they have fewer neighbours nearby, surface molecules share stronger attractive forces and bind together more tightly. This enhanced attraction results in tension that minimizes the total surface area.

Adhesion is a molecular attraction among dissimilar molecules. In this case, water interacts with a solid substrate, such as the glass walls of a capillary tube or the cell walls of tracheids or vessel elements-through hydrogen bonding. Water molecules are pulled upward as they bond to each other and adhere to the side of the tube.

Cohesion is a molecular at action among like molecules, such as the hydrogen bonding that occurs among molecules in water. Because water molecules cohere, the upward pull by adhesion is transmitted to the rest of the water column. The water column rises against the pull of gravity.

All three forces counteract the effect of gravity, and the result is capillary action, ensuring an unbroken column of water throughout the xylem vessels.

The effects of adhesion, cohesion, and gravity are responsible for the formation of a concave surface boundary called a meniscus (plural: menisci). A meniscus forms at most air-water interfaces-including those found in narrow tubes, such as xylem. Menisci form because adhesion

and cohesion pull water molecules up along 1e sides of the tube, while gravity pulls the water surface down in the middle. When adhesion creates an upward pull at the water-container interface, surface tension creates an upward pull all across the surface, and cohesion transmits both forces to the water below. All three forces counteract the effect of gravity, and the result is capillary action.

However, like root pressure, capillary action can transport water only a limited distance. This process moves water along the surfaces of mosses and other low-growing, non-vascular plants, but it can raise the water in the xylem of a vertical stem only about 1 m. Thus, root pressure and capillary action cannot explain how water moves from soil to the top of a redwood tree that can grow 5 to 6 stories higher than the Statue of Liberty. How then does it actually happen?

The Cohesion-Tension Theory

The leading hypothesis to explain long-distance water movement in vascular plants is the cohesion-tension theory, which states that water is pulled from roots to the tops of trees along a water-potential gradient, via forces generated by transpiration at leaf surfaces. This process relies on two of the forces involved in capillary action, namely, cohesion and tension. It operates as follows:

When a stoma opens, this humid air is exposed to the atmosphere, which in most cases is much drier. This contrast creates a steep water-potential gradient between the leaf interior and its surroundings. Water vapour evaporates from the sub-stomatal spaces of the spongy mesophyll cells due to heat from the sun leading to **transpiration**.

As water is lost from the leaf to the atmosphere, the humidity of the gas-filled space inside the leaf drops. In response, more water evaporates from the parenchyma cells in the leaf. This results in a reduced water potential in the cells next to the xylem. Water therefore enters these cells from the xylem sap which has a higher water potential.

Water molecules form **hydrogen bonds** between one another and hence tend to stick together with a force called **cohesion**. Water therefore forms a *continuous*, *unbroken column* across the mesophyll cells and down the xylem.

As water vapour evaporates from mesophyll cells in the leaf into the sub-stomatal air space, more molecules of water are drawn up behind it as a result of cohesion.

A column of water is eventually pulled up the xylem as a result of transpiration under a negative suction force. This is called **transpiration pull**.

The transpiration pull puts the xylem under tension, hence the name cohesion-tension theory.

Note: Because tracheids and vessels are dead at maturity, the water in xylem does not cross plasma membranes. As a result, water does not move between cells by osmosis. In xylem, water movement is driven entirely by differences in **pressure potential**.

The water in a column of xylem cells moves by bulk flow-a mass movement of molecules along a pressure gradient. The pulling force generated at menisci in leaf cell walls lowers the pressure potential of water in leaves. Even though the tension created at each meniscus is relatively small, there are so many menisci in the leaves of an entire plant that the tension created by summing many small pulling forces is remarkable. It creates a water-potential gradient between leaves and roots that is steep enough to overcome the force of gravity and pull water up long distances.
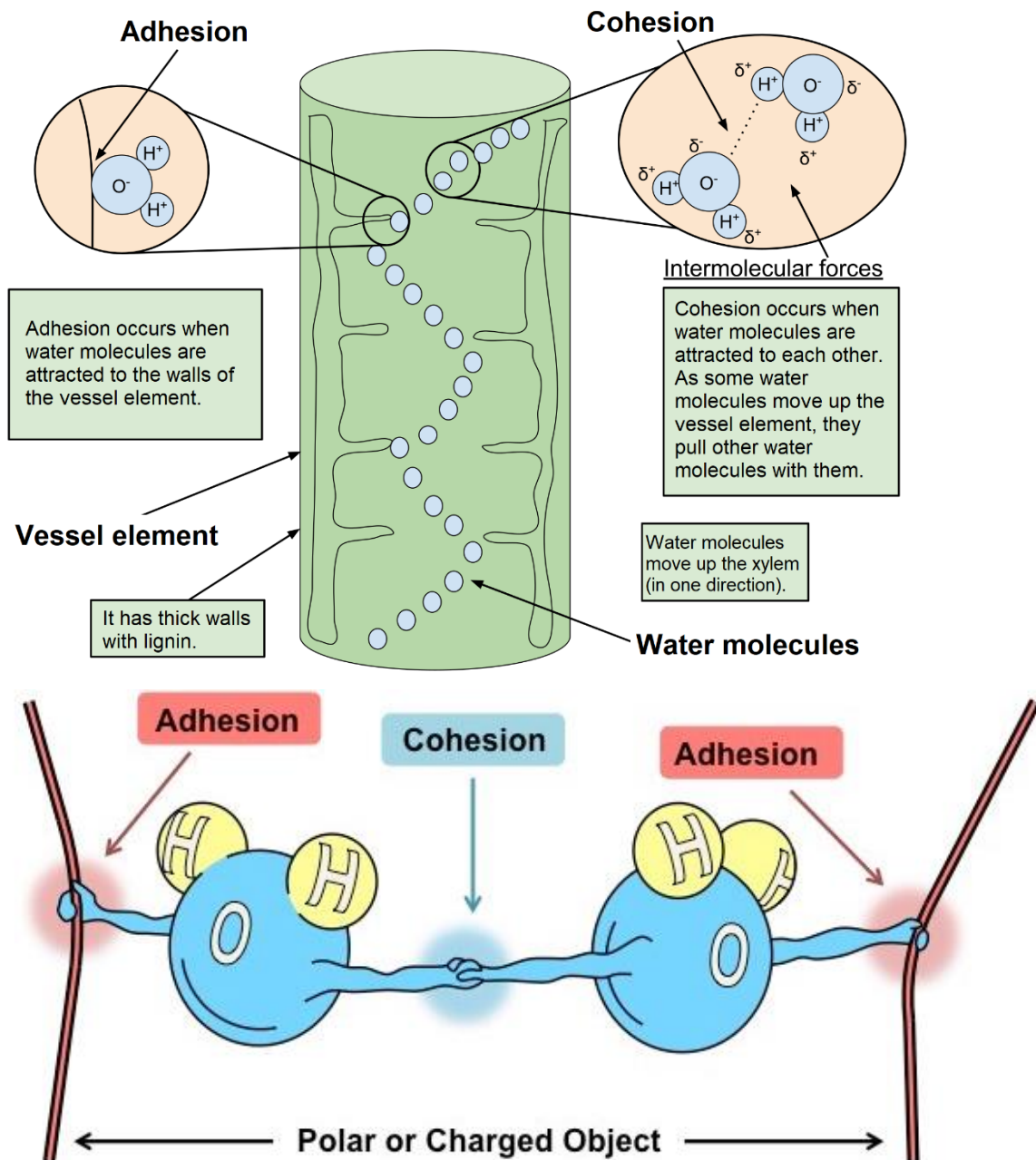
STRUCTURE AND FUNCTION XYLEM

Xylem is the main water conducting tissue in vascular plants. It also provides support for plants.

Structure of xylem

Xylem performs the functions of both supporting the plant and transporting water and minerals. Sclerenchyma fibres in the xylem all contribute to support, whereas the vessels and tracheids have support and transport roles.

Xylem fibres are elongated sclerenchyma cells with walls that are thickened with lignin; these features suit them for their support roles.

Xylem vessel elements vary in structure, depending on the type and amount of thickening of their cell walls, but are hollow and elongated. As they mature, their cell walls become impregnated with lignin, which causes them to die. The end walls break down, which allows the cells to form a continuous tube. (*The word 'element' is sometimes used rather than 'cell' because a cell is a living structure, whereas mature xylem vessels are dead*).

Sometimes, the lignin forms rings (annular thickening) around the vessel; in other cases, it forms a spiral or a network (reticulate thickening). This arrangement is better than a continuous thickening, because it allows elongation of the vessels as the plant grows, and allowing flexibility of plant parts during mechanism tension.

There are areas on the lignified wall where lignin is absent. These non-lignified regions are called **pits**. They are no completely open as there is still a cellulose cell wall across them – it is just that the wall is not lignified at these points. Pits allow for lateral (sideways) movement of water. In angiosperms (flowering plants), xylem vessels are the structures through which the vast majority of water is transported.

Figure 1 Xylem in LS as seen using a light microscope
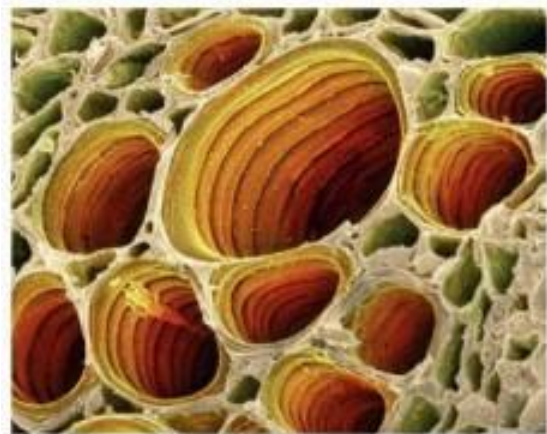
Figure 2 Leaf of tobacco showing xylem vessels (SEM) (×500 approx.)
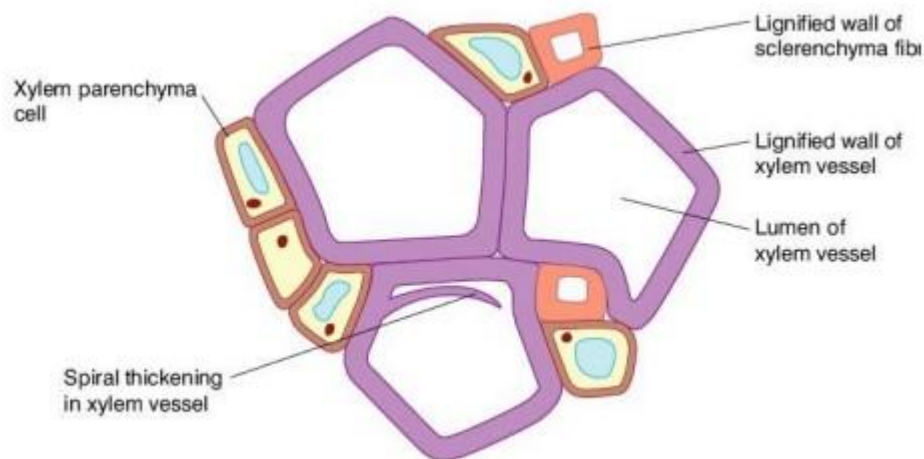
Figure 4 Xylem in TS as seen using a light microscope

Different types of thickening found in vessels

annular   Simple spiral   multiple spiral   reticulate
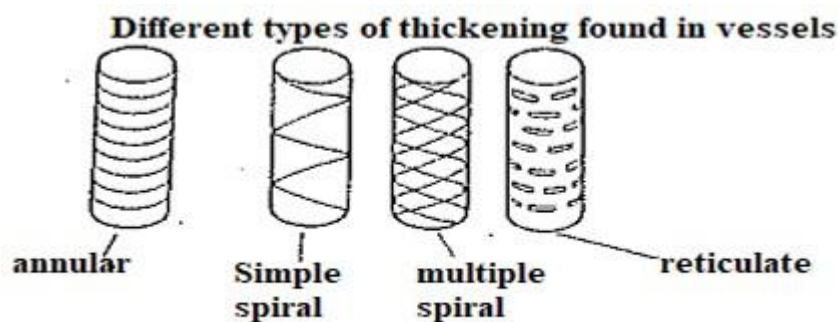
Adaptations of xylem to its function

1. The cells are long and arranged end to end to form a continuous tube

2. The cell dies when mature, which means that:
    - There is no cytoplasm or nucleus to hinder water flow
    - The end walls can break down, allowing a larger volume of water per unit time to be transported due to presence of a larger lumen

3. Even in tracheids where there is limited breakdown of the end walls are present, large bordered pits can allow exchange of water between neighbouring xylem cells ensuring continued flow of water.

4. Cell walls are thickened with lignin, which:
    - Makes them more rigid and therefore withstanding the great tension created by the transpiration pull.
    - Is waterproof to prevent water from escaping.

5. Annular, reticulate and spiral thickening allow xylem vessels to elongate during growth, and make them more flexible, so that branches can bend in the wind.

6. There are pits throughout the cells for lateral water flow, allowing supply of water to surrounding cells.

7. Cellulose walls increase the adhesion of water molecules, which helps to resist the effects of gravity and keep the column of water moving up.

8. The narrowness of the lumen of vessels and tracheids increases capillary forces causing a faster rate of water accent.

9. Possession of unspecialized parenchyma cells that act as packing tissue. These cells, roughly spherical in shape, surround other xylem components. When they become turgid, they exert pressure on one another, flattening in some areas. This close packing enhances their ability to provide structural support, ensuring that the xylem can withstand internal pressure and maintain the integrity of water transport throughout the plant.

TRANSLOCATION OF ORGANIC SOLUTES IN PHLOEM

Not all parts of a plant are photosynthetic. The main photosynthetic organs are the leaves. For those parts such as roots which are some distance from the sites of photosynthesis, there is a need for a transport system to circulate the products of photosynthesis. In vascular plants phloem is the tissue which carries products of photosynthesis away from the leaves to other parts.

In plants, that organic solutes (products of photosynthesis) must be able to move up and down in the same plant. This contrasts with movement in the xylem, which is only upwards.

Xylem and phloem are often found adjacent to each other, and both are specialized for transporting fluids. However, these two types of vascular tissue are radically different in their structure and in the way they transport fluids.

Translocation refers to the movement of sugars by bulk flow in multiple directions throughout a plant, but specifically, from sources to sinks. In vascular plants, a source is a tissue where sugar enters the phloem; a sink is a tissue where sugar exits the phloem

Note also that storage organs act either as sources (losing food) or as sinks (gaining food) at different times. Typically, about 90% of the total solute carried in the phloem is the carbohydrate **sucrose**, a disaccharide. This is a relatively inactive and highly soluble sugar, playing little direct role in metabolism and so making an ideal transport Sugar since it is unlikely to be used in transit.

Once at its destination sucrose can be converted back to the more active monosaccharides, glucose and fructose. Its high solubility means that it can be present in very high concentrations, up to 25% mass to volume of water in the phloem of plants such as sugarcane.

Phloem also carries certain mineral elements in various forms, particularly nitrogen and sulphur in the form of amino acids, phosphorus in the form of inorganic phosphate ions and sugar phosphates, and potassium ions. Small amounts of vitamins, growth substances such as auxins and gibberellins, viruses and other components may also be present.

Where do sources and sinks occur in a plant?

The answer often depends on the time of year.

•*During the growing season*: Mature leaves and stems that are actively photosynthesizing produce sugar in excess of their own needs. These tissues act as sources. Sugar moves from leaves and stems to a variety of sinks, where sugar use is high and production is low. Apical meristems, lateral meristems, developing leaves, flowers, developing seeds and fruits, and storage cells in roots all act as sinks.

• *Early in the growing season*: When a plant resumes growth after the winter or the dry season, sugars move from storage areas to growing areas. Storage cells in roots and stems act as sources; developing leaves act as sinks.

Structure of phloem

Phloem is composed of a number of cell types:

Sieve tube elements are elongated cells that are joined end to end to form long tubes. The cells are living and retain a thin layer of cytoplasm under their cell surface membrane (peripheral cytoplasm), which lies against the cellulose cell wall. In the cytoplasm are mitochondria and a modified form of endoplasmic reticulum. However, unlike most cells, there is no nucleus or Golgi body and there are no ribosomes. These structures are broken down in order to have fewer cell structures within the sieve tubes and so reduce resistance to the flow of materials within them. The end walls of sieve tubes are perforated by large pores, 2 - 6μm in diameter. These perforated end walls are called **sieve plates**. The central space within the sieve tube is called the **lumen**.

Companion cells are always associated with sieve elements and both come from the same cell division. As the sieve tube elements lack structures such as a nucleus, ribosomes and Golgi body, they are unable to carry out many metabolic processes essential for their survival. The companion cells are the site of these processes. With all the required organelles, dense cytoplasm and thin cellulose cell wall, they perform the metabolic activities for both themselves and the sieve tube elements. In the areas where assimilates are loaded or unloaded there are many plasmodesmata between the companion cell and the phloem sieve tube element.

At the tips of veins in the leaf, companion cells have very folded cell walls and cell surface membranes. These special types of companion cells are called **transfer cells** and their   large surface area increases the rate of transfer of sucrose into the sieve tube elements.
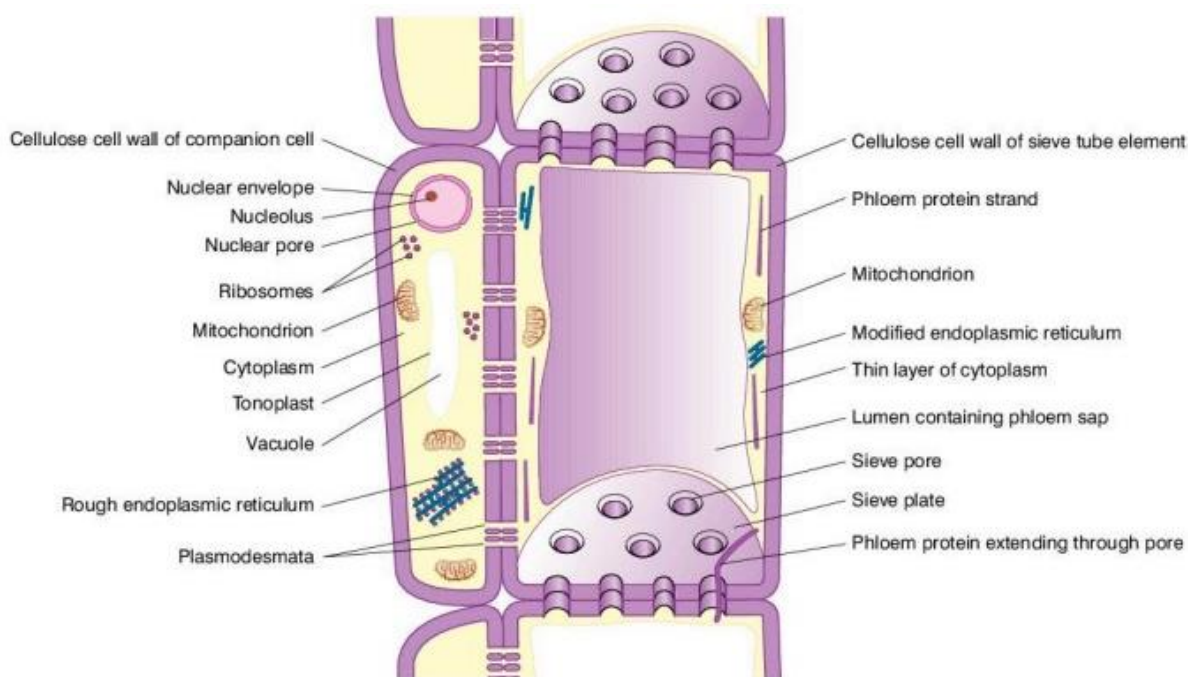
Figure   Sieve tube element and companion cell structure as shown by using an electron microscope

Adaptations of the phloem to its functions

The structure of sieve tubes has evolved to suit their function of transporting organic materials in solution.

1. Sieve tube elements are elongated and arranged end to end to form a continuous tube ensuring smooth continuous flow of food.

2. The nucleus and most of the organelles are located in the companion cells, leaving the lumen of the sieve tube elements more open, hence reducing resistance to the flow of liquid.

3. Sieve plates are perforated with sieve pore, reducing resistance to liquid flow.

4. Sieve plates hold the walls of sieve tube elements together and prevent them from bursting under great pressure.

5. The walls contain cellulose microfibrils that run around the cells, giving strength and preventing the tubes from bursting under pressure.

6. The walls are thin reducing the distance for faster entry of water at the source necessary for building the pressure.

7. Companion cells have many mitochondria to release the ATP needed for the active translocation of organic materials.

8. Plasmodesmata in the areas of loading and unloading allow easy movement of substances to and from companion cells.

9. Phloem proteins are a variety of different proteins that are thought to have a role in defense against pathogens and in sealing wounds.

Translocation of organic molecules in plants.

Having produced sugars during photosynthesis, the plant needs to transport them from the sites of production, known as **sources** (e.g. leaves and storage organs) to other parts of the plants, known as **sinks** (e.g. buds, flowers, fruits, roots and storage organs) where they will be used directly or stored for future use.

As sinks can be anywhere in a plant, sometimes above and sometimes below the source, hence translocation of molecules in phloem can be in either direction, transported in separate sieve tubes. The phloem transports sucrose and amino acids as well as inorganic ions such as potassium, chloride, phosphate and magnesium ions.

Mechanism of translocation

It is accepted that substances are transported in the phloem and that the rate of movement is too fast to be explained by just diffusion. The **mass flow hypothesis** (pressure flow hypothesis) is a widely accepted hypothesis to explain the mechanism of how translocation of assimilates is achieved. It can be divided into three phases:

A. *Loading: Transfer of sucrose into sieve tube elements from photosynthesising tissue.*

In contrast to the cohesion-tension model of water movement in xylem, pressure flow often requires that plants expend energy to set up a water-potential gradient in phloem. To establish a high pressure potential in sieve-tube elements near source cells, large amounts of sugar have to be transported into the phloem sap enough to raise the solute concentration of sieve-tube elements. One way that many plants appear to load sucrose into phloem sieve tubes is by a process known as loading:

o   Sucrose is manufactured from the products of photosynthesis in cells with chloroplasts.

o   Hydrogen ions (protons) are actively pumped from companion cells to their exterior, by proton pumps using ATP.

o   The hydrogen ion concentration builds up and these hydrogen ions then flow down their concentration gradient through carrier proteins (facilitated diffusion) into the cells.

o   Sucrose molecules are then transported along with the inflowing hydrogen ions in a process called **co-transport**. The carrier proteins are therefore known as **symporters**. This movement is against the concentration gradient for sucrose and is powered by the flow of protons back into the companion cell.

o   Sucrose molecules then move by diffusion through the plasmodesmata from the companion cell into the phloem sieve tube element.
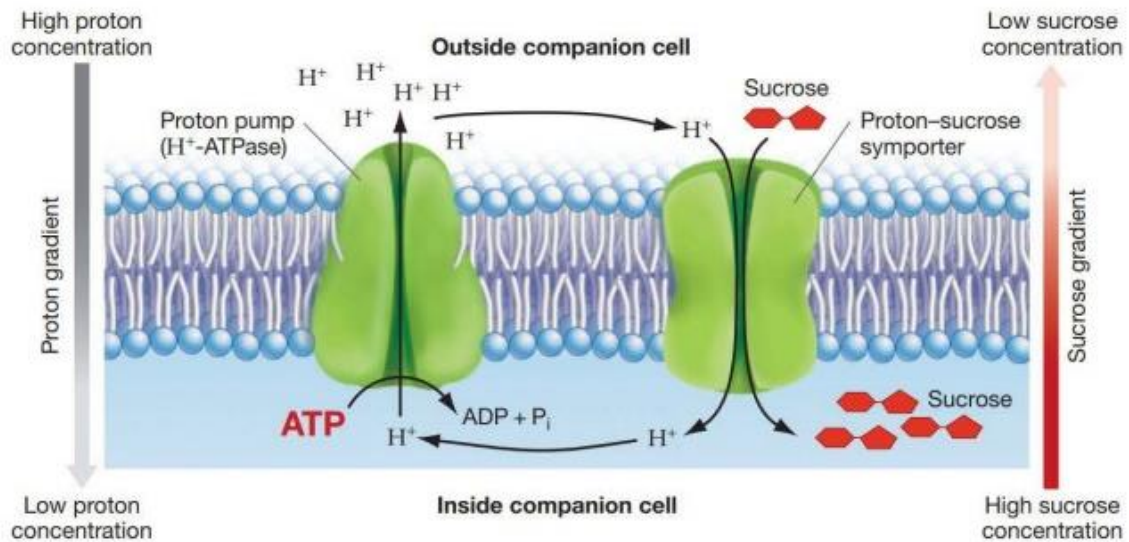
Figure: Cotransport during the loading process

B. Movement of phloem sap in sieve tube elements from **source to sink**

Different hypotheses have been suggested for the flow of sap through the phloem tubes and these include: *mass flow (pressure flow)*, *electro-osmosis* and *cytoplasmic streaming (transcellular strand hypothesis)*, and these are as discussed below.

1. Mass flow (pressure flow) hypothesis.

'Mass flow' is the bulk movement of substances though a channel under pressure gradients. Mass flow of sucrose through sieve tube elements takes place as follows:

Loading of sucrose at the source decreases the water potential of the sieve tubes at that section.

As the xylem has a much higher (less negative) water potential, water moves from the xylem into the sieve tubes by osmosis, this increases the volume of the solution, generating a high hydrostatic pressure within tube.

On the other hand, at the sinks (e.g. respiring cells and storage regions), sucrose is either used up during respiration or converted to starch for storage.

These cells therefore have a low sucrose content and so sucrose is constantly actively transported into them from the sieve tubes, and this lowers their (respiring cells') water potential.

This makes water in the phloem tube at that section to move into the surrounding sink cells by osmosis, while some water returns into the xylem adjacent to the phloem at that section, and this therefore decreases the hydrostatic pressure of the sieve tube element at that section (near the sinks).

As result of water entering the sieve tube elements at the source and leaving at the sink, there is a high hydrostatic pressure at the source and a low one at the sink.

There is therefore a mass flow of sucrose solution down this hydrostatic pressure gradient in the sieve tubes.

2. Electro-osmosis hypothesis.

Originally put forward by Spanner in 1958, and since modified on several occasions, the theory proposes that potassium ions are actively transported by companion cells, across the sieve plate.

This movement of ions draws polar water molecules across the plate. The movement is till one of mass flow, but the theory at least offers some function for the sieve plates and explains the high metabolic rate observed in companion cells. However, there is no consistent evidence of a potential different existing across sieve plates.

3. Cytoplasmic streaming.

Thaine in 1962 proposed that transcellular strands, which extend from cell to cell via pores in the sieve plate, carry out a form of cytoplasmic streaming. The solutes move in this cytoplasmic streaming which can occur between the strands or through them as they are in fact tiny tubules about 20nmm in diameter.

The process, being active, accounts for the many mitochondria in both sieve tube elements and companion cells. It will, however, require more positive proof of the existence of the actual process before it becomes widely accepted.

C. *Unloading: Transfer of sucrose from the sieve tube elements into storage of other sink cells.*

The sucrose is actively transported by companion cells, out of the sieve tubes and into the sink cells. (This occurs by a reversal of the events occurring during loading
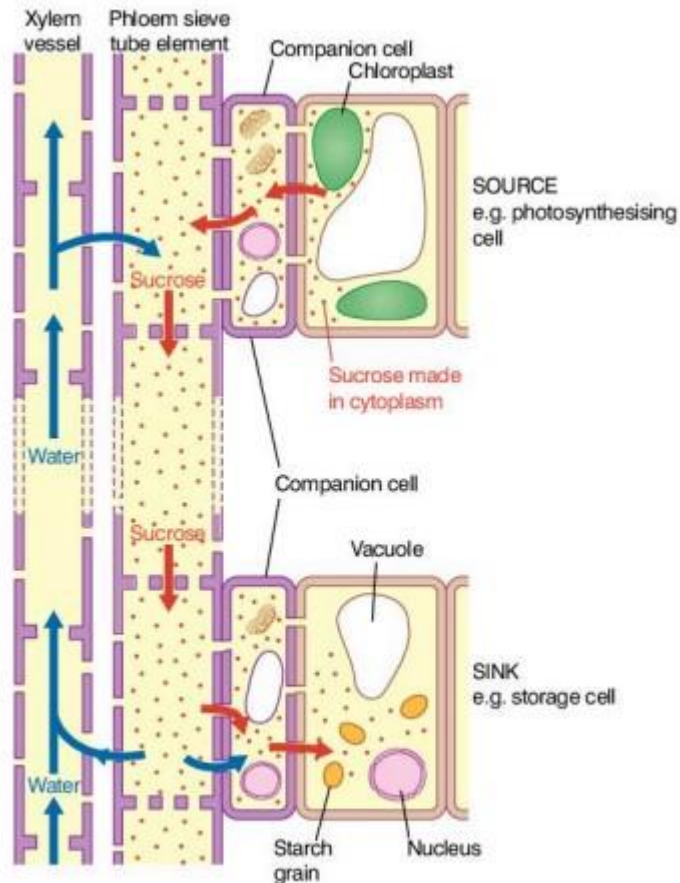
Figure Movement of sucrose from source to sink through the phloem of a plant

Munch's demonstration of the pressure flow hypothesis

Glass tube linking cell A to cell B represents the phloem

Direction of flow of sucrose solution

Cell A – has membrane permeable only to water. It contains a solution with a high concentration of sucrose. It represents the leaf of a plant

Cell B – has a membrane permeable only to water. It contains a solution with little sucrose. It represents respiring or storage regions of the plant e.g. roots

Water enters cell A by osmosis because it has a lower water potential than the water surrounding it

Water leaves because it is forced out due to the high pressure potential created in A

Direction of water flow

Glass tube linking the two vessels – represents the xylem

NOTES BY TR. BUUVI BRIAN   0756001375/0783662103

Evidence supporting mass flow hypothesis in the phloem

1. There is flow of solution from phloem when it is cut or punctured by the stylet of an aphid indicating flow of liquid under pressure.

2. When the phloem is cut, the sap which exudes out of it is rich in organic food materials especially sucrose and amino acids indicating flow of food through the phloem.

3. If the aphids are then severed from their stylets, sap continues to flow out through the stylets. This confirms that the aphids do not actively suck the fluid and therefore phloem sap is indeed under pressure, which forces it to enter the aphid

4. Occurrence of concentration gradients of sucrose and other materials, with high concentrations in the leaves and lower concentrations in roots.

5. Viruses of growth chemicals applied to leaves are only translocated downwards to the roots when the leaf to which they are applied is well illuminated and therefore photosynthesising.

6. Exposure of a plant to a respiratory poison like cyanide greatly slows phloem flow. This proves that mass flow is an active process requiring ATP from respiration.

7. In a ringing experiment, a complete ring of bark, which contains the phloem is removed from a tree trunk or stem. The tissue just above the ring swells, whereas that below the ring tends to wither. Analysis of the liquid within the swollen tissue shows that sugars and other organic solutes have accumulated above the ring. This suggests that removal of the phloem interrupts the downward movement of these substances, and that the tissues below the ring wither due to lack of nutrients.

8. When a plant is exposed to an atmosphere containing carbon dioxide labelled with radioactive carbon-14, ^{14}C, the sugars later found in the phloem contain carbon-14. When the phloem and the xylem are separated by waxed paper, the carbon-14 is found to be almost entirely in the phloem, indicating that the organic substances synthesised in the leaf are translocated in the phloem.

Criticisms on the pressure flow hypothesis

1. It offers no explanation for the existence of sieve plates which act as a series of barriers impeding flow. (Though one suggestion is that sieve plates provide mechanical support to the sieve tubes, preventing them from buckling or splitting when they carry sap at high hydrostatic pressure, and may also be involved in repairing leaky sieve tubes, or isolating a sieve tube that has burst open.

2. Since the process is passive after the initial stage, there is no necessity for the phloem to be a living tissue at all.

3. While the theory would suggest that all materials being transported in the phloem would travel at the same speed, in practice sugars and amino acids move at different rates.

TRANSPIRATION

Transpiration in plants is the process where water is absorbed by roots, transported through the plant, and then released as water vapour from the leaves, primarily through tiny openings called stomata. This process is crucial for nutrient transport, temperature regulation, and maintaining turgor pressure within plant cells.

Types of Transpiration

These are based on the sites through which water is lost from the plant, and are as follows;

1. Stomatal Transpiration: The primary type, where water evaporates water from cells and diffusion of the water vapour through stomata, the pores found in the epidermis of leaves and green stems. About 90% of the water is lost this way.

2. Cuticular Transpiration: A small amount of water loss occurs by evaporation of water from the outer walls of epidermal cells through the waxy cuticle covering the epidermis of leaves and stems. About 10% of the water lost, varying with thickness of cuticle.

3. Lenticular Transpiration: This involves water loss through lenticels, which are small openings in the stems and bark of trees for gas exchange. Only minute amounts of water are lost this way, although this is the main method of water loss from deciduous trees after leaf fall.

Importance of Transpiration

- Cooling: Transpiration aids in regulation of plants' temperature. As water evaporates from the leaf surfaces, it absorbs heat, cooling the plant and preventing overheating, especially in hot conditions.

- Nutrient Transport: Creates a suction force known as the transpiration pull, which draw water and dissolved minerals from the roots through the xylem vessels to the leaves and other parts of the plant.

- Maintaining turgidity: By aiding continuous water flow through the plant keeping the cells turgid, this enables support to the leaves and stems especially in herbaceous plants.

Factors affecting the rate of transpiration.

External Factors

1. Temperature: An increase in temperature raises the kinetic energy of water molecules within the plant. This causes the water molecules to move faster, which increases the rate of evaporation from the surfaces inside the leaves and through the stomata. Higher temperatures also cause the air around the plant to hold more water vapour, making the air less saturated, which further drives transpiration as the plant loses more water to the drier surrounding air.

2. Humidity: Humidity refers to the amount of water vapour present in the air. When humidity is high, the air around the leaves is already saturated with water, reducing the concentration gradient between the moist leaf interior and the air outside. This slows down the rate of water vapour diffusion out of the leaf, thus decreasing transpiration. In contrast, low humidity increases the concentration gradient, promoting faster water vapour movement out of the leaf, leading to a higher transpiration rate.

3. Wind Speed: Wind removes the humid air layer around the leaf surface, maintaining a low water vapour concentration in the surrounding air. This keeps the concentration gradient high, so water vapour continues to diffuse out of the leaf. In still or stagnant air, water vapour accumulates around the leaf, reducing the concentration gradient and thus slowing transpiration. However, very strong winds may cause stomata to close as a protective response to prevent excessive water loss, thereby reducing transpiration.

4. Light Intensity: Light stimulates the opening of stomata to allow carbon dioxide in for photosynthesis. Open stomata allow water vapour to escape, increasing the rate of transpiration. In low light conditions, such as at night, stomata usually close to conserve water, thereby reducing the transpiration rate.

5. Soil Water Availability: When soil water is plentiful, roots absorb it readily, and the plant maintains normal transpiration rates. If water in the soil is limited, plants may experience water stress, leading them to close their stomata to prevent water loss. This slows down transpiration, helping the plant conserve water during droughts.

Internal Factors

1. Leaf Surface Area: Larger leaves have more stomata and surface area for water to evaporate from, which can increase the transpiration rate. Plants adapted to dry environments often have smaller leaves or needle-like structures to reduce water loss and lower the transpiration rate.

2. Number and Distribution of Stomata: The more stomata a leaf has, the more pathways are available for water vapour to escape, increasing transpiration. Stomata on the lower surface of the leaf (common in many plants) experience less direct sunlight and, thus, lower evaporation, helping to regulate water loss. This adaptation minimizes excessive transpiration.

3. Cuticle Thickness: The cuticle is a waxy layer on the surface of the leaf that acts as a barrier to water loss. A thicker cuticle reduces water evaporation from the leaf surface, thereby decreasing transpiration. Plants in dry environments often have a thicker cuticle to conserve water.

4. Leaf Structure and Orientation: Leaves that are oriented to minimize direct exposure to sunlight experience lower temperatures on their surfaces, which reduces the kinetic energy of water molecules and slows transpiration. Some plants have leaf hairs (trichomes) that trap moisture near the leaf surface, reducing the concentration gradient and slowing down water vapour diffusion, and also capture air around the leaf which insulates the leaf from high environmental temperatures thus decreasing transpiration.

Experiment: To investigate and measure factors affecting rate of transpiration using a potometer

A potometer is a piece of apparatus designed to measure the rate of water uptake by a cut shoot or young seedling. It does not measure transpiration directly, but since most of the water taken up is lost by transpiration, the two processes are closely related.

Materials

1. A potometer, which comprises of a conical filter flask, short rubber tubing, rubber bung with a single hole, hypodermic syringe and needle, graduated capillary tube.
2. Large black polythene bag.
3. Large transparent polythene bag.
4. Small electric fan.
5. Retort stand and clamp.
6. Stop clock.
7. Thermometer.
8. Vaseline (petroleum jelly).
9. Leafy shoot such as lilac bucket.

Method

1. Select a suitable leafy plant, cut off the shoot and immerse the cut end immediately in a bucket of water to minimise the risk of air being drawn into the xylem. Immediately cut the shoot again under water, with a slanting cut, a few centimetres above the original cut. The stem must be thick enough to fit tightly into the bung of the potometer.

2. Submerge a conical filter flask in a sink of water to fill it with water. Transfer the leafy shoot from bucket to sink and again immediately make a slanting cut a few centimetres above the last cut. Fit the shoot into the bung of the flask under water and push the bung in to make a tight fit.

3. Submerge the graduated capillary tube, with rubber tubing attached, in the sink, fill it with water and attach it to the side arm of the filter flask.

4. Remove the apparatus from the sink and set up the syringe with the needle pushed into the rubber tubing. The syringe can be clamped in a vertical position. The joint between shoot and bung should be smeared with vaseline to make certain it is airtight.

5. As the shoot takes up water, the end of the water column in the capillary tube can be seen to move. It may be returned to the open end of the tube by pushing in water from the syringe. Allow the shoot to equilibrate for 5min while regularly replacing the water taken up.

6. Measure the time taken for the water column to move a given distance along the capillary tube and express the rate of water uptake in convenient units, such as cm/min. A number of readings should be taken, to ensure that the rate is fairly constant, and the mean result calculated. The temperature of the air around the plant should be noted.

(7) Each time the air bubble reaches the end of the graduated section of the tube return it to its original position with the syringe.

(8) The effects of some of the following factors on rate of uptake of water could be investigated:

(a) Wind — use a small electric fan (do not strongly buffet the leaves or the stomata will close).
(b) Humidity — enclose the shoot in a transparent plastic bag.
(c) Darkness — enclose the shoot in a black polythene bag.
(d) Removal of half the leaves — is the transpiration rate halved?
(e) Vaselining upper and/or lower epidermises of the leaves to prevent water loss. In each case sufficient time should be allowed to ensure that the new rate has been attained. It is not always possible to change only one condition at a time; for example, enclosing the plant in a transparent bag will also lead to some reduction in light intensity.

Absolute rate of water uptake

Results can be converted to actual volume of water taken up per unit time, such as cm^3/h, if the volume of the graduated scale corresponding to each division is determined. Most of the water taken up is lost through the leaves. An estimate of rate of water loss per unit leaf area can be obtained by measuring the volume of water lost as described above and then removing all the leaves and determining their surface area. The latter can be obtained by drawing the outlines of the leaves on graph paper and counting the enclosed squares. Using these data, results can be expressed as $cm^3/h/m^2$ leaf area.

Results

The results of the experiment should reveal that the plant's transpiration rate is influenced by various environmental factors. Analysis of the data suggests that increased temperature and air movement enhance the plant's water uptake, while high humidity slows it down.
Conversely, darkness significantly reduces the plant's water absorption.
Suggest an effect of removing half the leaves!
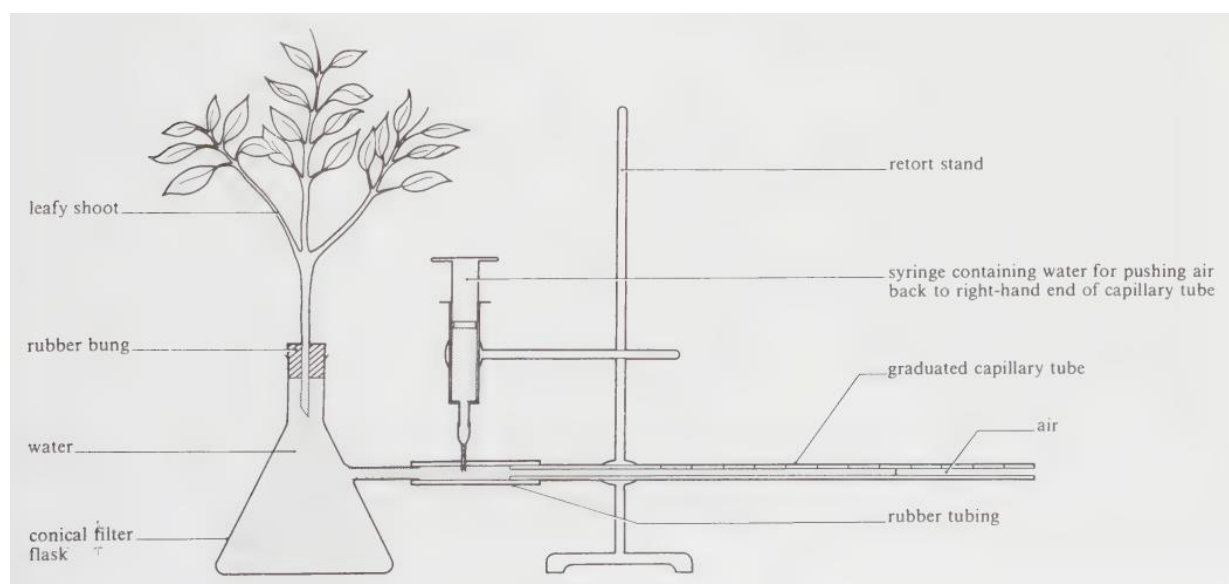
MECHANISM OF OPENING AND CLOSING OF THE STOMATA

Starch-sugar interconversion hypothesis:

Daytime Process (Stomatal Opening)**:** During the day, photosynthesis in the guard cells consumes CO_2, leading to an increase in pH. This higher pH activates the enzyme phosphorylase, which converts starch into soluble sugars. The progressive formation of sugars increases the concentration of these soluble sugars which raises the osmotic concentration within the guard cells. As a result, water enters the guard cells through endosmosis, making them turgid. The turgid state of the guard cells causes them to swell and bend, resulting in the opening of the stomatal pore.

Nighttime Process (Stomatal Closing)**:** At night, photosynthesis ceases, leading to the accumulation of CO_2 in the guard cells and a subsequent drop in pH. This lower pH inactivates the phosphorylase enzyme, causing the soluble sugars to be converted back into starch. This conversion reduces the osmotic concentration of the guard cells. Consequently, water exits the guard cells through exosmosis, making them flaccid. The flaccid state of the guard cells causes them to shrink, resulting in the closing of the stomatal pore.

Photosynthetic product theory:

During daylight, guard cells, which contain chloroplasts, carry out photosynthesis, producing sugars. The accumulation of these sugars increases the osmotic pressure of the cell sap, causing water to move into the guard cells from neighbouring epidermal cells by osmosis. This influx of water results in the expansion and increased turgidity of the guard cells, leading to the opening of the stomata.

In darkness, photosynthesis ceases, and the sugar in the guard cells is converted to starch, lowering the osmotic pressure of the guard cells. Consequently, water moves out of the guard cells to neighbouring cells by osmosis, causing the guard cells to become flaccid and the stomata to close. However, this theory does not fully explain how the low rate of glucose formation can account for the rapid opening of stomata.

Potassium ion transport theory:

Daytime Process (Stomatal Opening): This theory suggests that during day, hydrogen ions build up inside guard cells from sources like the decomposition of malic acid (malate + H^+) formed starch in presence of light. Concurrently, light also activates proton pumps, pump hydrogen ions out of the guard cells, increasing the negative charge inside the cells (hyperpolarization). To balance the charge, potassium ions (K^+) enter the guard cells by diffusion from surrounding cells. Chloride ions also follow potassium ions into the guard cells so as to balance the charge. The accumulation of K^+, malate and Cl^- lowers the water potential of the guard cells. As a result, water enters the guard cells through endosmosis, making them turgid and more curved, causing the stomata to open.

Nighttime Process (Stomatal Closing): At night, H^+ ions move back into the guard cells from surrounding cells down their concentration gradient, and K^+ ions and Cl^- exit the guard cells to balance the charge. H^+ ions react with malate to form malic acid, reducing the osmotic concentration of the guard cells. Consequently, water exits the guard cells through exosmosis, making them flaccid and less curved, causing the stomata to close.

Apparently, darkness during the night promotes the biosynthesis the hormone abscisic acid (ABA), increasing its concentration. ABA binds to receptors at the cell membrane of the guard cells, increasing the permeability of calcium channels. Calcium ions (Ca^{2+}) enter the guard cells, triggering the release of Ca^{2+} from the vacuole into the cytosol. This influx of Ca^{2+} and the movement of K^+ ions, along with certain organic ions like malate, out of the guard cells collectively increase the water potential in the guard cells, leading to water diffusion out to neighbouring cells by osmosis. As the turgor pressure decreases, the guard cells become flaccid and the stoma closes.

NB: The potassium ion transport hypothesis demonstrates the need of ATP in the closing and opening of the stomata, hence the process can be greatly affected by respiratory inhibitors.

However, ABA production can also be triggered by water stress, which is a condition where a plant loses more water by transpiration than it absorbs it from the soil.