1 Transpiration

Transpiration is defined as the **loss of water** from the plant in the form of water **vapor**. It can be assumed that the driving **force** for transpiration is the **difference in water potential** between the **sub-stomatal air space** and the **external atmosphere.**

In leaf mesophyll there is an extensive **system of intercellular spaces** which **correspond** to the **internal surface** of water contact **with the air**. By this interface between the cell walls and the intercellular spaces is established a **water potential gradient**. Due to the **water evaporation in the surface** of the cell walls which are in contact with the air in the intercellular spaces, it is established that the **tensile** (stretchy) strength is **transmitted to the xylem** that drives the **upward flow** of the water column **from the root** and is produced in the internal evaporation process in the leaves. The **water vapor** moves from **leaf** intercellular spaces **to the atmosphere** predominantly through **stomatal diffusion**. This process of the loss of water vapor by the leaves is called transpiration and corresponds to the majority (90%) of the volume of water absorbed by plants.

Transpiration may be considered a two-stage process:

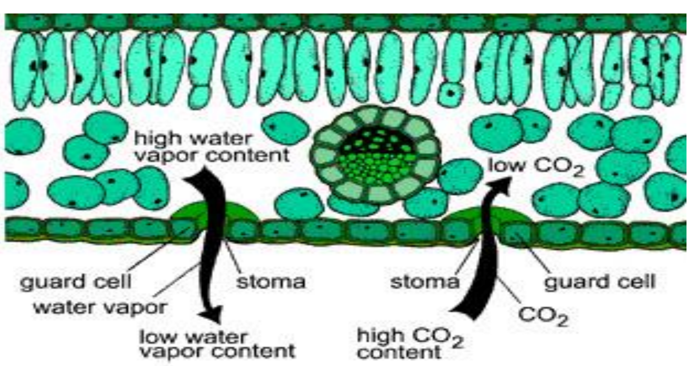
(1) The evaporation of water from the moist cell walls into the sub-stomatal air space.

It is commonly assumed that evaporation occurs primarily at the surfaces of those **mesophyll cells** that border the sub-stomatal air spaces.

(2) The diffusion of water vapor from the sub-stomatal space into the atmosphere.

Once the water vapor has left the cell surfaces, it diffuses through the sub stomatal-space and exits the leaf through the **stomatal pore**.

In principle we can assume that the **sub-stomatal** air space of leaf is normally **saturated** with **water vapor.** On the other hand, the atmosphere that **surrounds the leaf** is usually **unsaturated** and may often have very **low water content**. This **difference in water vapor pressure** between the internal air spaces of the leaf and the surrounding air is the **driving force of transpiration**.



1.1 Types of transpiration:

1. **Stomatal** transpiration; accounts for **90 to 95%** of water loss from leaves.
2. **Cuticular** transpiration; accounts for the remaining **5 to 10%**.

3. **Lenticular** transpiration; a small amount of water vapor may be lost through small openings called (**lenticels**) in the bark of young twigs and branches,

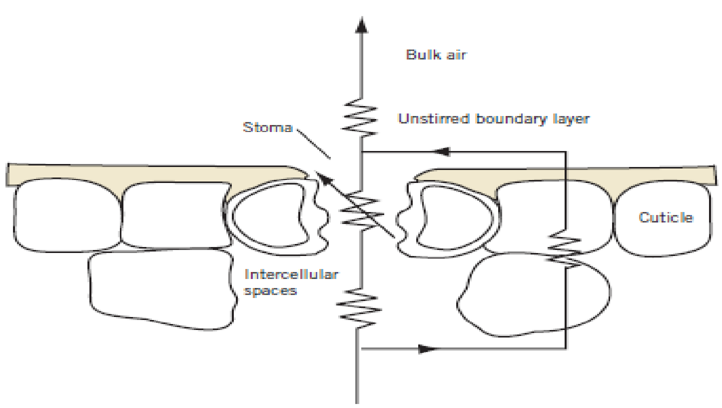
In most **herbaceous species**, stomata are present in both the upper and lower surfaces of the leaf, usually **more abundant on the lower surface**. In many **tree species**, stomata are located **only on the lower surface** of the leaf.

1.2 Transpiration from the leaf depends on two major factors:

1. The **difference in water vapor concentration** between the leaf air spaces and the external bulk air. **Fick's law** of diffusion tells us that the rate of diffusion is proportional to the **difference in concentration** of the diffusing substance. It therefore follows that the rate of transpiration will be governed in large measure by the magnitude of the vapor pressure difference between the leaf and the surrounding air.

2. The **diffusional resistance** of this pathway; The air space in the leaf is close to water potential equilibrium with the cell wall surfaces from which liquid water is evaporating. The concentration of water vapor changes at various points along the transpiration pathway from the cell wall surface to the bulk air outside the leaf.

The **diffusional resistance** of the transpiration pathway, consists of two varying components:
1. **Leaf stomatal resistance**; the resistance associated with diffusion through the stomatal pore.
2. **Leaf boundary layer resistance**; the resistance due to the **layer of unstirred air next to the leaf surface** through which water vapor must diffuse to reach the turbulent air of the atmosphere.



Various **anatomical** and **morphological** aspects of the leaf can influence the **thickness of the boundary layer**:

1. **Hairs** on the surface of leaves can serve as microscopic windbreaks.
2. Some plants have **sunken stomata** that provide a sheltered region outside the stomatal pore.
3. The **size and shape of leaves** also influence the way the wind sweeps across the leaf surface.

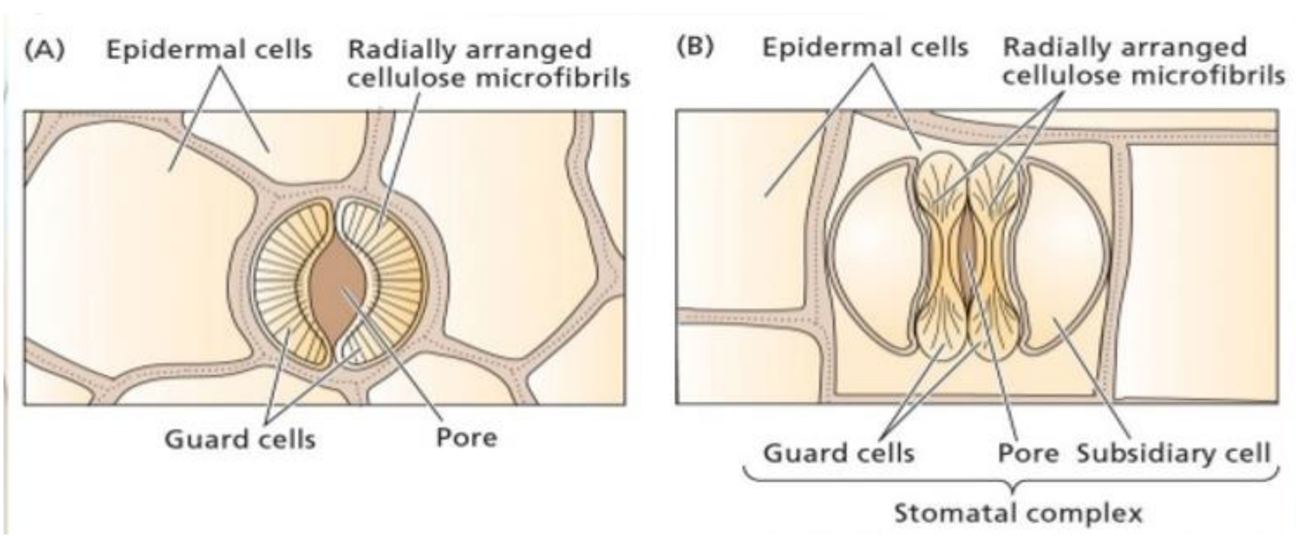
4. To change the **orientation of their leaves** and thereby influence their **transpiration rates.**
5. Many grass **leaves roll up** as they experience **water deficits**, in this way increasing their **boundary layer resistance**.

1.3 Stomatal apparatus

The epidermis of leaves contains pores that provide for the exchange of gases between the internal air spaces and the ambient environment. This minute opening is called stomata (singular stoma). Stomata occur in the epidermis of all parts of the shoot system, even in flower parts such as stamens and pistils. The opening, or stoma, is bordered by a pair of unique cells called guard cells. In most cases the guard cells are in turn surrounded by specialized, differentiated epidermal cells called **subsidiary cells**. Subsidiary cells provide a reservoir of water and ions that move into and out of the guard cells as they change shape during stomatal opening and closing.

Guard cells are crucially important functional elements: they regulate stomatal apertures, thereby controlling rates of CO2 uptake and water loss and hence influencing photosynthesis and water status of the plant. Stomata are especially numerous on the lower epidermis of horizontally oriented leaves—an average of about 100 stomata per square millimeter, although the actual number varies widely—and in many species are located only on the lower surface. The lower epidermis of apple (Malus sylvestris) leaves, for example, has almost 400 stomata per square millimeter, whereas the upper epidermis has none. This adaptation reduces water loss, in part because stomata on the lower epidermis are shielded from direct sunlight and are therefore cooler than those on the upper epider

The alignment of cellulose microfibrils, which reinforce all plant cell walls and are an important determinant of cell shape, plays an essential role in the opening and closing of the stomatal pore. In ordinary cells having a cylindrical shape, cellulose microfibrils are oriented transversely to the long axis of the cell. As a result, the cell expands in the direction of its long axis because the cellulose reinforcement offers the least resistance at right angles to its orientation.



In **grasses**, guard cells have a characteristic **dump bell shape**, with bulbous ends. These guard cells are always flanked by a pair of differentiated epidermal cells called **subsidiary cells**, which **help the guard cells** control the stomatal pores.

In **dicots** and **non-grass monocots**, guard cells have an elliptical contour (often called "**kidney-shaped**") with the pore at their center. **Subsidiary** cells are often **absent**; the guard cells are **surrounded by ordinary epidermal cells**. A distinctive feature of guard cells is the specialized structure of their walls.

At night, when there is **no photosynthesis** and thus **no demand for CO_2** inside the leaf, stomatal **apertures** are kept **small** or **closed**, **preventing** unnecessary **loss of water**. Leaf can regulate its stomatal resistance by opening and closing of the stomatal pore. This biological control is exerted by a pair of specialized epidermal cells, the **guard cells**, which surround the stomatal pore.

1.4 Factors affecting opening and closing of stomata:

1. **Light**: Among external factors, light plays predominant role in the movement of guard cells. **Blue** and **red** light are effective in both **photosynthesis** and **stomatal opening**. However, **blue** light is found to be more effective (relative to red light) in **causing stomatal opening** than in photosynthesis. At **low light** levels, **blue light** may cause stomatal opening when red light has no effect at all. Blue light causes **movement of K+.** It is now thought that blue light promotes the **breakdown of starch into the PEP molecules** that can accept CO2 producing malic acid.

2. **Water Content of Epidermal Cells**: The movement of guard cells is **turgor phenomenon** and guard cells derive water from the adjoining epidermal cells and hence water content of the latter is bound to affect this phenomenon.

3. **Temperature**: **Increase in the temperature** causes stomata to **open**. Temperature has significant effect on the **permeability** of the wall of the guard cells and therefore greatly affect the osmotic phenomenon which is responsible for the movement of these cells.

4. **Mineral Elements:** Deficiency of certain mineral elements like nitrogen, phosphorus and potassium has some effect on the opening and closing of stomata (Desai, 1937).

1.5 Mechanism of Stomatal Opening and Closing

Opening and closing of stomata takes place due to changes in turgor of guard cells. Generally, stomata are open during the day and close at night. The actual mechanism responsible for entry and exit of water to and from the guard cells has been explained by several theories. These theories are described below:

A. **Proton - Potassium Pump Hypothesis:** Levit in 1974 combined the points in Scarth's and Steward's hypothesis and gave a modified version of the mechanism of stomatal movement which was called the proton - potassium pump hypothesis. According to this hypothesis **K+ ions are transported into the guard cells** in the presence of **light**.

During Day Light	During Night/Dark
Accumulation of K^+ ions by the guard cells	Loss of K^+ ions by the guard cells
↓	↓
Increased solute concentration	Decreased solute concentration
↓	↓
Endosmosis of water	Exosmosis of water
↓	↓
Increased turgidity	Decreased turgidity
↓	↓
Stoma opens	Stoma closes

The uptake of K+ ions is balanced by one of the following:

1. Uptake of chloride (Cl^-) ions as anions. The subsidiary cells lack chloroplast and take up Cl– ions as anions to balance the influx of K+ ions.
2. Transport of H^+ ions released from organic acids. In some plants the guard cells contain starch, there is accumulation of organic acid like malate by conversion of starch into malic acid in light. The organic acid dissociates into malate and H+. Potassium reacts with malate to form potassium malate which increases the solute concentration.
3. Entry of K^+ is balanced by exit of protons (H^+).

B. The Starch - Sugar Interconversion Theory: This theory was put forward by Steward in 1964. According to him, during **day time** phosphorylase ***enzyme converts the starch into sugar*** due to which **osmotic potential of guard cell increased** and allow the entry of water into the cell. During the **night** same reaction occur in **reverse direction** which closes the guard cell i.e. stomata is closed during night.

Reaction in Light

Utilization of CO_2 during photosynthesis in guard cells

↓

Drop in CO_2 leads to increase in pH or protoplasm becoming alkaline

↓

Conversion of starch into sugar

↓

Increased concentration of solute

↓

Endosmosis of water from subsidiary cells to the guard cells

↓

Increased turgor pressure of the guard cells leads to turgidity of guard cells accompanied by flaccidity of subsidiary cells

↓

Stoma opens

↓

A. Stoma opens during light

Reaction in Dark

Accumulation of CO_2 due to absence of photosynthesis in guard cells

↓

Increased acidity or decrease in pH due to formation of carbonic acid

↓

Conversion of sugar into starch

↓

Decreased concentration of solutes in guard cells

↓

Exosmosis of water from guard cells to subsidiary cells

↓

Decreased turgor pressure in guard cells leads to flaccidity of guard cells, accompanied by increased T.P. and turgidity of subsidiary cells.

↓

Stoma closed

↓

B. Stoma closed at dark

C. Synthesis of organic solutes: Blue light also stimulates the **starch degradation** and **malate biosynthesis**. Malate is an organic acid. In plants, malate is synthesized in the guard cell cytosol form the compound generated from the hydrolysis of starch. The enzyme phosphoenol pyruvate carboxylase (PEP carboxylase) binds carbon dioxide to PEP to produce oxaloacetate, which is then reduced to malate and stored in the vacuole.

D. Role of Abscisic Acid (ABA) It has been observed that during water shortage in the soil or by intense solar radiation, a plant hormone abscisic acid accumulates in the leaves leading to closing of stomata, thus preventing an excessive water loss. Under experimental conditions also, when abscisic acid is applied to the leaves, stomata get closed and check water loss

In the presence of light, guard cells also perform photosynthesis. It increases the osmotically active solutes such as sucrose. Thus, in the presence of **light**, concentration of **K+, Cl-, malate** and **sucrose increases**. The increase in these osmotically active substances in the guard cells causes osmotic potential (**Ψs**) increase and water to move passively into these cells and as their turgidity increase, the stomatal pore opens.

As **Ψs increases**, the **water potential decreases**, and water consequently moves into the guard cells. As **water enters** the cell, **turgor pressure increases**. Because of the **elastic properties** of their **walls**, guard cells can reversibly **increase their volume** by **40 to 100%**, depending on the species. Such **changes** in cell **volume** led to **opening or**

closing of the stomatal pore. Subsidiary cells appear to play an important role in allowing stomata to open quickly and to achieve large apertures.

The opening or closing of stomata occur in response to signals from the external environment.

- Light = Stomata open
- Dark = Stomata close
- High CO2 inside leaf = stomata close
- Low CO2 inside leaf = stomata open
- Drought stress = stomata close

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

The effectiveness of plants in **moderating water loss** while allowing **sufficient CO_2 uptake** for photosynthesis can be assessed by a parameter called the **transpiration ratio**. This value is defined as the amount of water transpired by the plant divided by the amount of carbon dioxide assimilated by photosynthesis.

For plants in which the first stable product of carbon fixation is a 3-carbon compound (**C3 plants**), as many as **400- 500 molecules of water are lost every molecule of CO_2 fixed** by photosynthesis, giving a **transpiration ratio of 400**.

Plants in which a 4-carbon compound is the first stable product of photosynthesis (**C4 plants**), generally transpire **less water** per molecule of CO_2 fixed than C3 plants do. A typical **transpiration ratio** for C4 plants is about **150**.

Plants with crassulacean acid metabolism **(CAM)** photosynthesis the transpiration **ratio is low**, values of about **50** are not unusual.

1.7 The Environmental Factors Affecting Transpiration:

1. Light. Light, specifically **light intensity**, is probably the most obvious among the environmental factors **affecting** transpiration in plants. It has a controlling effect on the **opening** of the stoma through which water primarily escapes in gaseous state. In general, **transpiration rate is high** during **daytime**, particularly when light is bright, than during night time.

The stomata are typically **open** during daytime, allowing the **entry of CO_2** and the **exit of O_2**. The opening of the stomata likewise enables the escape of water as water vapor in the process of **stomatal transpiration**. Except in **CAM plants**, the stomata are close at darkness between sunsets to sunrise. In turn, photosynthesis decreases the concentration of CO2 in the intercellular spaces within the leaf resulting to the opening of the stomata.

2. Relative Humidity. At **high RH** (moist air), the **stoma** tends to **close** and thus limit the exit of water vapor from the plant. Further, high RH means that the **water-potential**

gradient (also water vapor concentration and vapor pressure gradient) from plant to the atmosphere will be **minimal** compared to when RH is low. In addition, at high RH the atmosphere contains more water and has low atmospheric demand, meaning that it has **limited capacity** to absorb more water.

At **50% relative humidity** at a temperature of **20°C,** water potential **Ψw** of the atmosphere is **-93.5 MPa** but at **90% RH**, water potential will be **-14.2 MPa**.

On the other hand, the typical water potential of the leaves of a **small tree** that grows with **sufficient soil moisture** will be **-1.5MPa**. In both RH, transpiration occurs whereby water vapor moves outward from higher to lower water potential or from less negative to more negative water potential values, i.e.,

- From Ψw = -1.5MPa to Ψw = -93.5 MPa (at **50% RH**).
- From Ψw = -1.5MPa to Ψw = -14.2 MPa (at **90% RH**).

Transpiration rate will be **faster at 50%** than at 90% RH because;

At 50% RH, the water potential gradient is steeper (gradient); (93.5 MPa – 1.5 MPa = **92 MPa**) compared to **90% RH** (14.2 MPa - 1.5 MPa = **12.7 MPa**).

But, at low RH also favors **faster transpiration** due to **stronger atmospheric demand**. But as long as the stomata are open, transpiration occurs, even at saturated condition of 100% RH. In this case the expelled water vapor readily condenses.

Note: The water potential of pure water in an open container is zero because there is no solute and the pressure in the container is zero. Adding solute lowers the water potential. When a solution is enclosed by a rigid cell wall, the movement of water into the cell will exert pressure on the cell wall.

3. Temperature. The rate of transpiration is **fastest** when air temperature is between **20°C to 30°C**. At these temperatures the **stomatal apertures** or opening are generally **widest**. In general, the stomata **close** at temperatures about **0°C** and progressively increase in aperture up to about **30°C**.

Temperature as an environmental factor affecting transpiration also relates to water potential and relative humidity. The relationship of temperature (T, in °K), relative humidity (RH, in %) and water potential (Ψw, in pascal) is shown in the following mathematical equation provided by Hopkins (1995):

$$\Psi w = 1.06\ T \log (RH/100)$$

Applying the equation, **an increase in temperature** will **decrease water potential**. Thus at the same RH of 50%, Ψw at temperature of 20°C or 293.15°K is -93.5 MPa but lower (more negative, Ψw = -96.7 MPa) at higher temperature of 30°C. Increase in atmospheric temperature will therefore steepen further the plant-air water potential gradient.

4. Soil Water. Where the **supply of water** from the soil is **limiting**, the rate of transpiration tends to **slow down**. This is more pronounced where other conditions, such as bright light and warm temperature, favor escape of water from the plant. In this case, water deficit within the plant may occur leading to the **closing of the stomata** which is manifested by **wilting of leaves**. Conversely, the entry of **water into the guard cells** opens the stoma. This allows the **passage of water** in the process of stomatal transpiration.

5. Wind. This environmental factor affects transpiration by **removing** that **thin moist layer of air,** called **boundary layer**, which lies next to the surface of a leaf. This moist air causes a lesser water potential gradient from the leaf resulting to reduced rate of transpiration. This layer also reduces **light penetration** into the leaf. **But with wind**, this boundary layer is replaced with **drier air** thus increasing water potential gradient and enhancing transpiration. However, **strong wind** may cause **excessive loss** of water from leaves leading to **stomatal closure**.

1.8 Internal plant factors affect transpiration rate

Certain plant adaptations reduce transpiration:

- **Reduced size of the leaves**, thereby reducing transpiring surface. Some xerophytic plants have needle like or spine like leaves (Pinus and Opnuntia)

- **Thick deposition of cutin** (wax like substance) on the leaf surface.

- **Stomata found sunken** in the cavities surrounded by epidermal hairs as in Nerium and Cycas.

- **Root shoot ratio**, when there is more root and less of shoot system or leaves, there will be more of transpiration. Root is the water absorbing surface and shoot or leaves represent the transpiring surface; high root shoot ratio will cause more transpiration.

1.9 Significance of transpiration

Transpiration has a number of positive effects:

1. Absorption of water. Transpiration pull influences the rate of absorption of water from the soil.

2. Water movement. By transpiration, water moves upwards and as it passes into the cell vacuoles, it makes the cells turgid. This gives a form and shape to the cells and to the plants as a whole.

3. Mineral salt transport. The water stream moving upwards also carries the dissolved minerals required for the development of the plant. Transpiration also helps in distributing these minerals throughout the plant body.

1.10 Antitranspirants:

A number of substances are known which when applied to the plants retard their transpiration. Such substances are called as Antitranspirants. Some examples of Antitranspirants are:

1. Colorless plastics, silicone, oils, low viscosity waxes and phenyl mercuric acetate, etc. Colorless plastic, silicone oils and low viscosity waxes belong to one group as these are sprayed on the leaves, form after film which is permeable to O_2 and CO_2 but not to water.
2. Fungicide phenyl mercuric acetate, when applied in low concentration (10-4 m), it exercised a very little toxic effect on leaves and resulted in partial closure of stomatal pores for a period of two weeks.
3. ABA a plant hormone also induces stomatal closure. CO_2 is an effective Antitranspirants.
4. A little rise in CO_2 concentration from the natural 0.03% to 0.05% induces partial closure of stomata. Its higher concentration cannot be used which results in complete closure of stomata affecting adversely the photosynthesis and respiration.