Aim

To study the structure of stomata and determine stomatal density on upper and lower surfaces of leaves.

Introduction

Stomata: Microscopic pores on leaf surfaces, regulated by guard cells.
Function: Facilitate gas exchange (CO_2 intake) and transpiration (water loss).
Objective: Measure stomatal density using a microscope and study stomatal structure.

Materials Required:

- Plant Leaves (Tradescantia or Pancratium or Hibiscus)
- Microscope slides and cover slips
- Transparent nail paint
- Transparent sticky tape
- Glycerine
- Safranin
- Razor blade
- Forceps
- Microscope
- Digital camera or smartphone
- Clear plastic ruler (metric scale)

Protocol:

Step 1: Prepare Materials
Collect all required materials.
Select a healthy, fully expanded leaf for analysis.
Step 2: Leaf Preparation
Cut the leaf obliquely into two pieces.
Place each piece in separate watch glasses containing distilled water:
One for the upper (adaxial) surface
One for the lower (abaxial) surface
3: Peel Preparation
Using forceps, carefully peel the upper and lower epidermal layers.
Mount each peel on a clean glass slide with a drop of glycerine.
Add a drop of diluted safranin to stain the stomata.
Method: Stomatal Impression Technique Using Nail Polish and Sticky Tape
1: Selection of Leaf Samples
Select fresh, healthy leaves from a normal (unstressed) plant.
Select fresh leaves from a plant exposed to stress conditions (e.g., water stress, high light, or salinity).
Ensure that leaves are clean and free from dust.
2: Application of Nail Polish
Place the leaf flat with the lower (abaxial) surface facing upward.
Apply a thin, even layer of clear nail polish on the lower surface of the leaf using a brush.
Allow the nail polish to dry completely for 10–15 minutes until a transparent film is formed.
3: Preparation of Stomatal Impressions
Place a small piece of transparent sticky tape over the dried nail polish film.

Protocol: (cont)

Press gently to ensure proper adhesion between the tape and the nail polish layer.

Carefully peel off the sticky tape using forceps; the nail polish film containing stomatal impressions will be transferred onto the tape.

4: Mounting of Impression

Place the sticky tape with the nail polish impression onto a clean glass slide.

Add a drop of water or glycerine if required to improve clarity.

Gently place a cover slip over the tape to avoid air bubbles.

Step 4: Microscopic Observation

Observe the mounted preparation under the microscope using low power (10×) first.

Switch to high power (40×) for detailed observation of stomata.

Record observations on the size, shape, distribution, and density of stomata.

Compare stomatal features between healthy and stressed plants.

Step 5: Microscopic Observation

Place a cover slip over the mounted peel.

Use the transparent ruler method to measure the field of view (FOV):

Place a clear plastic ruler on the microscope stage under the stage clips.

Rotate to the lowest magnification objective (4×).

Focus using coarse, then fine adjustment until metric markings are clear.

Align the ruler to measure the diameter of the circular field of view (record in mm).

Calculate the radius:

Radius (r)=Diameter (D)/ 2

For 10× and 40× objectives, calculate the FOV using:

$FOV_{low} \times Mag_{low} = FOV_{high} \times Mag_{high}$

Calculate the area of the FOV:

$Area=\pi r^2$

$\pi=3.14$

Step 6: Calculate Stomata per 1 mm²

Stomata per 1 mm^2= Number of stomata counted / Area of field of view (mm^2)

Step 7: Stomatal Structure Analysis

• Examine the size, shape, and distribution of stomata under the microscope.

• Note any differences between upper and lower surfaces.

• Capture images for documentation.

Observation Table:

Calculation of Field of View (FOV) and Area

Objective Magnification	Diameter of FOV (mm)	Radius of FOV (mm) (D/2)	Area of FOV (mm²)
4X			
10X			
40X			

Table 2

Plant Name	Objective Magnification	Leaf Surface	Stomatal Density (Stomata/FOV area mm²)	Average Stomatal Density (Stomata/FOV area mm²)	Stomatal Density (Stomata / 1 mm²)
	10X	Upper	FOV1 Reading.....		
			FOV2 Reading.....		
			FOV3 Reading.....		
		Lower	FOV1 Reading.....		
			FOV2 Reading.....		
			FOV3 Reading.....		

Result

The stomatal density of Plant leaf was determined using microscopic observation. The upper (adaxial) surface showed an average stomatal density of ..... stomata/mm², while the lower (abaxial) surface had a higher density of .....stomata/mm².