

### 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<sub>2</sub> 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.



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### 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:

$$\text{Radius (r)} = \text{Diameter (D)} / 2$$

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

$$\text{FOV}_{\text{low}} \times \text{Mag}_{\text{low}} = \text{FOV}_{\text{high}} \times \text{Mag}_{\text{high}}$$

Calculate the area of the FOV:

$$\text{Area} = \pi r^2$$

$$\pi = 3.14$$

#### Step 6: Calculate Stomata per 1 mm<sup>2</sup>

$$\text{Stomata per 1 mm}^2 = \text{Number of stomata counted} / \text{Area of field of view (mm}^2\text{)}$$

#### 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 (mm <sup>2</sup> ) |
|-------------------------|----------------------|--------------------------|--------------------------------|
| 4X                      |                      |                          |                                |
| 10X                     |                      |                          |                                |
| 40X                     |                      |                          |                                |



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**Table 2**

| Plant Name | Objective Magnification | Leaf Surface | Stomatal Density (Stomata/FOV area mm <sup>2</sup> ) | Average Stomatal Density (Stomata/FOV area mm <sup>2</sup> ) | Stomatal Density (Stomata / 1 mm <sup>2</sup> ) |
|------------|-------------------------|--------------|------------------------------------------------------|--------------------------------------------------------------|-------------------------------------------------|
|            | 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/mm<sup>2</sup>, while the lower (abaxial) surface had a higher density of .....stomata/mm<sup>2</sup>.



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