WJEC Wales Biology A Level

SP 2.2a: Investigation into stomatal numbers in leaves

Practical notes

Introduction

Stomata are pores found on the surface of leaves, typically the **lower epidermis**. They open and close, with the aid of **guard cells**, to **control gas exchange** and **water loss**.

Stomatal density varies between the upper and lower epidermis and from species to species. It can be investigated using the following method.

Equipment

- Leaves
- Fine forceps
- Scissors
- White tile
- Clear nail varnish
- Microscope
- Eyepiece graticule
- Stage micrometer
- Microscope slide
- Coverslip
- Dropping pipette
- Distilled water

Risk assessment

Hazard	Risk	Precaution	Emergency
Broken glass	Cuts	Keep glassware away from the edge of the desk; handle microscope slides carefully	Dispose of broken glassware carefully; elevate cuts; do not remove glass from cuts; seek medical assistance
Scissors	Cuts	Handle carefully; keep scissors away from the edge of the desk	Elevate cuts and apply pressure; wash minor cuts in cold water; seek medical assistance
Nail varnish	Allergy	Use PVA glue alternative; wear non-latex disposable gloves when handling nail varnish; wash hands thoroughly after experiment	Seek medical assistance

Plant sap	Allergy	Wear non-latex disposable gloves; wash hands thoroughly after experiment	Seek medical assistance

Method

Preparing epidermal impression

1. Position a leaf on a white tile with its **lower epidermis** facing **upwards.**
2. Coat the leaf surface with a layer of **clear nail varnish**. Leave to dry for a couple of minutes before applying a **second coat**.
3. Use a pair of **fine forceps** to carefully **peel** away the nail varnish layer. This produces an **impression** of the lower epidermis.
4. Whilst using a pair of fine forceps to hold the sample, cut to an appropriate size to fit the microscope slide. Place the sample in the centre of a **microscope slide**.
5. Use a pipette to add **2 drops** of **distilled water** onto the sample and apply a **cover slip**. *Lower the cover slip at an angle to prevent the formation of bubbles.*
6. Absorb any **excess** water on the microscope slide using a paper towel.

Using light microscope

1. **Calibrate** the microscope for all three objective lens magnifications (see 'Calibration of a light microscope' practical).
2. Place the microscope slide under the clips on the microscope stage and observe the impression using the **×40** objective lens.
3. Total the **number of stomata** in the **field of view**. Repeat for a further two fields of view and calculate a **mean**. Record your results.
4. Use the calibrated eyepiece graticule to measure the **diameter** of the **field of view** in **mm**. Calculate the **area** of the field of view using πr^2.
5. Calculate the **stomatal density** using:

$$\text{mean number of stomata per mm}^2 = \frac{\text{mean number of stomata per field of view}}{\text{area of field of view (mm}^2\text{)}}$$

Example results

Total number of stomata in field of view			
Area 1	Area 2	Area 3	Mean
6	5	3	4.67

For example if the field of view has a diameter of 0.45 mm:

Area = $0.225^2 \times \pi = 0.159$ mm^2

$$\text{mean number of stomata per mm}^2 = \frac{\text{mean number of stomata per field of view}}{\text{area of field of view (mm}^2\text{)}} = \frac{4.67}{0.159} = \mathbf{29.4}$$