Investigation into stomatal numbers in leaves

Specification reference:

AS Component: 2.3 **A level Component: 3.1**

Adaptations for gas exchange

Introduction

Stomata are pores surrounded by two guard cells on the aerial parts of plants. They are most densely packed in leaves, which therefore make suitable experimental material. This technique describes how to measure their density in the lower epidermis of a leaf.

Apparatus

Leaves
White tile
Fine forceps
Fine scissors/scalpel
Clear nail varnish/PVA glue
Microscope slides
Cover slips
Dropping pipette
Distilled water
Microscope

Method

1. Making a replica of the epidermis:

(i) Place a leaf on a white tile with its lower epidermis facing upwards.

(ii) Stretch the leaf between two fingers of one hand. With the other hand, apply a layer of colourless nail varnish between the veins, and allow it to dry.

(iii) Apply a second layer of nail varnish/PVA glue and allow it to dry.

(iv) Hold a pair of fine forceps horizontally and insert one point between the epidermis and the nail polish/PVA glue layer. Grip the layer and peel it away from you, maintaining tension in the peeled layer. This produces a replica of the lower epidermis.

(v) Place the replica on a microscope slide and use scissors/scalpel to cut a sample, taking care that the replica does not fold.

(vi) Apply two drops of water and cover with a cover slip.

2. Counting the stomata:

(i) Focus on the replica using the x10 objective and then refocus using the x40 objective.

(ii) Count the number of stomata in the field of view.

(iii) Repeat for three fields of view and calculate a mean.

3. Calculating stomata distribution:

(i) From your microscope calibration, calculate the area of the field of view by:

- Measuring the diameter of the field of view
- Converting to an actual size in mm
- Using πr^2 to calculate the area of the field of view

(ii) Calculate the distribution of stomata where

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

Risk assessment

Hazard	Risk	Control method
Leaves, depending on species can be toxic	Could be transferred from hand to mouth	Avoid ingesting
Dissecting instruments can be sharp	Could Pierce or cut skin when cutting sample	Handle with care

Teacher/ Technician notes

- Fully-expanded leaves are used. Leaves grow by cell expansion, rather than by cell division, so the number of stomata generally remains constant throughout the leaf's development. Their separation, and therefore, their density, is not fixed until the leaf is fully expanded.

- A stoma may be only partially visible. Consequently, it is useful to make a rule concerning stomata that are only partially within the field of view eg they will be counted if more than half the area of the stoma is visible, or they if appear in the top half of the field of view, but not the lower half.

- When making successive counts on a single slide, care must be taken to count a stoma only once. It is useful to move the slide on the stage in an organised fashion e.g. always to the right.

Sample results

Field of view	Number of stomata	Mean number of stomata
1	5	$\frac{37}{10}$ = 3.7
2	4	
3	2	
4	2	
5	5	
6	3	
7	4	
8	3	
9	4	
10	5	

Using a x10 objective, the field of view area is likely to be 0.4 mm^2.

Stomatal density = mean number of stomata per mm^2

$$= \frac{mean\ number\ of\ stomata\ per\ field\ of\ view}{area\ of\ field\ of\ view\ in\ \mathrm{mm^2}}$$

$$= \frac{3.7}{0.1} = 37.0\ mm^{-2}.$$

Further work

- Repeat the procedure with different leaves, such as a xerophyte eg *Kalanchoë*, a hydrophyte eg *Nymphaea* and a plant that evolved in the presence of plenty of water, such as *Ficus*.
- Compare the distributions on the lower epidermises of these plants and account for the differences, in view of the characteristics of the habitats in which they evolved.
- Compare distributions on the upper and lower epidermis of these plants and account for the differences, considering the arrangement of the leaves on the plant.
- Compare with the distribution on the two epidermises of cereal leaves, such as maize or barley and relate them to the growth habit of the leaves.

Practical Techniques

- use of light microscope at high power and low power, including use of a graticule