

# TEACHER/LECTURER GUIDE

## Type and purpose of activity

This experiment can be used to:

- provide evidence for assessment of Outcome 3. N.B. This is only possible if stomatal widths are measured using eyepiece graticules. Otherwise, the experiment is an illustrative practical. (For advice on marking Outcome 3 report, please contact SAPS Scotland)
- develop good microscope skills
- develop knowledge and understanding of how osmosis and the state of turgor in guard cells affects the opening and closing of stomata
- develop problem solving skills and in particular Outcome 2 PC:  
(d) Experimental procedures are planned, designed and evaluated appropriately.

## Background information

- *Commelina communis* provides an excellent epidermal peel. It is invariably one cell thick while the chloroplasts inside the guard cells are clearly visible. It should also provide a rare opportunity for students to see open stomata. The experiment clearly demonstrates that when guard cells are turgid stomata are open and when guard cells are flaccid stomata are closed.
- To make the results quantitative eyepieces need to be fitted with a graticule which then allows students to measure stomatal width in graticule units. (Hence the need for eyepiece graticules if Outcome 3 is to be assessed).
- Students need to have a firm grasp of the direction of water movement by osmosis when the percentage water bathing plant cells is changed. They also need to be familiar with the terms - turgor, turgid and flaccid.
- They may also be confused by the apparent contradiction that as guard cells lose water stomata close. This principle is well demonstrated by pumping up a bicycle tyre tube fully and then folding it in half. The distance between the two halves will gradually decrease as the tube is deflated.

## Reference

Weyers, J.D.B.(1994) Investigating stomatal physiology with epidermal strips from *Commelina communis* L (Dayflower)., *Journal of Biological Education*, **28**(4), 255-259.

## Classroom management

The lower epidermal peel is to be placed in three liquids - distilled water, 5% sucrose solution and 20% sucrose solution. Each student should be responsible for the preparation of at least one microscope slide.

To obtain good results it is necessary to observe the following points:

- the microscopes must be clean and be capable of x 400 magnification
- it is probably best to demonstrate how to make an epidermal peel
- students may also require help using the eyepiece graticule
- to measure stomatal width it will be necessary to move the slide and rotate the eyepiece so that the graticule scale lies above the stomata (see diagram in Student Activity Guide)
- cross contamination between the three liquids must be avoided
- the peel must be transferred quickly to the bathing liquid. Otherwise, stomata will close regardless.

## Supply of materials

In order to satisfy the core skill in problem solving, students will be required to 'identify and obtain resources' required for themselves. It is therefore not appropriate to provide all equipment and materials in e.g. a tray system for each student/group. Normal laboratory apparatus should not be made available in kits but should generally be available in the laboratory. Trays could be provided containing one type of specialist equipment or materials.

## Extension work

- Bathe epidermal peels in buffered solutions of different pH.
- Compare upper and lower epidermal peels.
- Compare responses of epidermal peel in other plants.
- The role of potassium ions in stomatal opening e.g. bathe illuminated epidermal peels with closed stomata in 150 mM KCl, NaCl and LiCl.
- Bathe epidermal peels in solutions of abscisic acid - a hormone involved in the closing mechanism.

# TECHNICAL GUIDE

## Materials required

### Materials required by each student/group:

microscope with x40 objective lens (with eyepiece graticule if Outcome 3 is being assessed)  
suitable illumination for microscope  
3 microscope slides  
coverslips  
10 cm<sup>3</sup> distilled water  
10 cm<sup>3</sup> 5% sucrose solution  
10 cm<sup>3</sup> 20% sucrose solution  
dropper  
scalpel  
seeker

### Materials to be shared:

*Commelina* plants enclosed in polythene bags under strong illumination  
Bicycle tyre tube and pump (optional)

## Preparation of materials

- Strong *Commelina* plants, about 40-70 days old should be put in bright light for 1-2 hours enclosed in a polythene bag. Their stomata should then open. Plants left in this situation for four hours or more may have closed stomata. Plants that are flowering will also give poor results.
- Plants will grow well in any good compost and especially under a light bank.
- Seeds should be sown at least 40 days before the plants are required in the classroom. They take about 14 days to germinate but do not require any special attention. The plants will eventually produce small blue flowers and set seed. These seeds can be easily collected and after a six month dormancy period be used to provide further plants.
- Eyepiece graticules available from:
  - Graticules Ltd., Morley Road, Tonbridge, Kent TN9 1RN Tel 01732 35906 19 mm diameter type NE1
  - A pack of 10 plastic graticule scales are available from Philip Harris

## PREPARING FOR THE ACTIVITY

Read through the Student Activity Guide and consider the following questions.

### Analysis of activity

Ensure you know the meaning of the following terms: osmosis; turgid; flaccid; turgor.

What will happen to the turgor of plant cells when bathed in (i) distilled water (ii) 20% sucrose solution?

What is the aim of the experiment?

What is being varied in the activity?

What variables must be kept constant?

What measurements are you going to make and how will you make them?

### Getting organised for experimental work

In your groups decide how the activity will be managed by allocating tasks to each member. For outcome 3 it is important that you play an active part in setting up the experiment and in collecting results.

### Recording of data

Prepare a table to record your results. You should use a ruler, correct headings and appropriate units.

### Evaluation

How will you avoid cross contamination between the three bathing media even if you have only one dropper available?

How can you perhaps avoid air bubbles on your prepared slide? Will you include stomata enclosed in air bubbles in your results?

How will you ensure that the epidermal peel is immersed as quickly as possible in the bathing medium? What will happen to the stomata if there is a delay?

When choosing stomata to measure their width why is it important to choose them randomly?

# STUDENT ACTIVITY GUIDE

## Background information

Since leaf surfaces are covered with a waxy cuticle, plants must use stomata to exchange gases with the atmosphere. However, water vapour also escapes when stomata are open and plants must therefore control stomatal opening to prevent excess water loss. They do this by varying the turgor of the guard cells.

In this experiment changes in turgor are brought about by bathing lower epidermal peels in solutions of different osmotic strengths. The resulting exchange of water between the bathing medium and the guard cells changes the turgor in these cells resulting in changes to the size of the stomata.

The plant used in this practical is *Commelina communis* L. (common name - day flower). It has been under bright light enclosed in a polythene bag for about two hours. The high light intensity and high humidity result in a high transpiration rate with stomata wide open.

## Equipment and materials

### Materials required by each student/group:

- 1 microscope with x40 objective lens
- 1 eyepiece fitted with a graticule - if Outcome 3 is being assessed. (A graticule is a microscopic scale, fitted into the eyepiece, which allows the relative size of an object to be measured under the microscope)
- Suitable illumination for the microscope
- 3 microscope slides
- 3 coverslips
- 10 cm<sup>3</sup> distilled water
- 10 cm<sup>3</sup> 5% sucrose solution
- 10 cm<sup>3</sup> 20% sucrose solution
- 1 dropper
- 1 scalpel
- 1 seeker

### Materials to be shared:

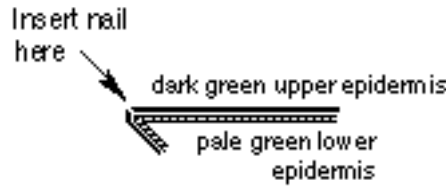
- Commelina* plants
- 1 bicycle tyre tube and pump (optional)

## Instructions

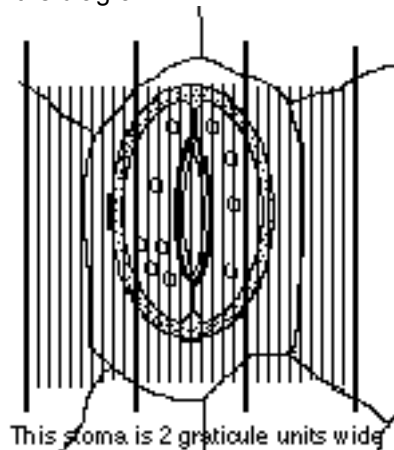
1. Collect the materials indicated above.
2. Set up three microscope slides with a large drop of distilled water, 5% sucrose solution or 20% sucrose solution on each one.
3. Select a leaf from a *Commelina* plant and use the scalpel to cut out a piece of leaf about 5mm wide and 20 mm long, avoiding the midrib, as shown in the diagram.



4. Carefully fold the piece of leaf near one end so that the upper (darker green) surface is on the outside of the fold. This should leave a small split on the upper surface of the leaf.



5. Insert your nail gently into the split and carefully peel away the upper layers of the leaf to expose the transparent lower epidermis
6. Immediately, place the lower epidermis on the microscope slide containing the appropriate solution.
7. Using the scalpel remove any green areas of leaf attached to the peel and if necessary reduce the size of the peel so that it will fit comfortably under the coverslip.
8. Ensure the peel is immersed in the solution on the slide. You may have to add more solution to achieve this.
9. Using a seeker lower a coverslip on top of the peel and gently blot to remove excess liquid.
10. Focus with a low power objective lens.
11. When you have located a suitable part of the slide, view under high power. If an eyepiece graticule is available measure the width of 10 stomata chosen at random. Move the slide carefully and rotate the eyepiece so the graticule scale sits across the stoma as in the diagram.



12. Examine the epidermal peels bathed in the other solutions and again measure the width of any 10 stomata.
13. Complete a table of results, using correct headings and appropriate units, recording the width of all stomata measured in your group. Calculate the average stomatal width for each slide.

### Abscisic Acid

This hormone linked with stomatal closing can be obtained from:

- A) Sigma (Tel. 0800 373731). Cat. no. A 7383.
- B) Philip Harris (Tel. 0141 952 9538). Cat. no. H74030/9.

A solution of 13 mg/250 cm<sup>3</sup> should close stomata. The water must be heated to almost boiling to get the abscisic acid into solution.

### Varying pH

To vary pH use different combinations of 0.1 M citric acid and 0.2 M disodium hydrogen phosphate as detailed for the catechol oxidase in banana experiment.