Expt 1. A Strategy to Survive on Dry Land

Leaf pores can open and close: guard cell movement and stomatal aperture

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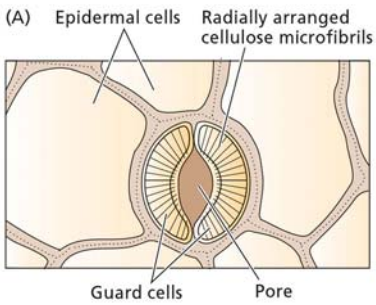

Objectives:

- Understand how plants survive on dry land.
  Plants need to pull water up from roots to leaves.
  Plants also take up CO_2 from the air for _____. .
- Observe how guard cell movement regulates the opening of stomata
- Test how environmental signals (like light and dark) regulate stomatal pore size
- Test the effect a plant stress hormone, abscisic acid, on stomatal pore. What do you hypothesize?
- Present your results to the class. Interpret. What do results mean?

Introduction

Pores of leaf surfaces are critical for pulling up water from roots to leaves by a process called transpiration. These pores also allow CO_2 entry into leaf for photosynthesis. However, water can escape from open pores leading to dessication. Thus plants need to control the opening and closing of the pores or stomatal aperture. In order to optimize photosynthesis and reduce water loss the aperture is regulated by the movement of two guard cells. The turgor pressure in leaf guard cells is used to open and close the stomatal opening. Environmental (e.g. light) and chemical signals cause ion movements that result in an increase of solutes in the guard cells. For example, day light stimulates the plasma membrane H^+-pumping ATPase, causing a change in the membrane potential which leads to the influx of K^+. The increase in solute concentration causes water to follow. The turgor increase stretches the cells on one side only, thus opening the stomates. These pressures can be very high; ranging upwards to 25 or 30 atm. During periods of water stress, stomatal apertures decrease and close to reduce water loss. The response to water stress is mediated in part by hormones, like ABA. ABA levels in plants increase when plant is drought-stressed.

Aim: Test the effect of light and dark on stomatal opening and test the requirement for K^+.
Test the effect of a stress hormone, abscisic acid, ABA.

Approach:

Epidermal strips taken from leaves of *Vicia faba* (fava bean) are exposed to various treatments, and observed under a microscope to determine the extent of stomatal closing or opening.

Reagents

100 mM KCl	500 ml	200 mM mannitol (250 ml)
100 mM choline Cl	250 ml	100 mM KCl with 10 uM ABA (250)

Supplies & Equipment	Microscopes ; slides & cover slips
100 mm petri plates (~ 50)	Digital camera attached to microscope
Forceps,	2-4 Flood lamps to mimick sunlight

Procedure:

Part I. What signals and ions alter guard cell movement? Why?

1. Prepare five petri plates with the following solutions:

dark (control)	a. 100 mM KCl	
light	b. 100 mM KCl	e. 100 mM KCl & 10 uM ABA
light	c. 200 mM mannitol,	
light	d. 100 mM choline Cl,	

2. Remove a healthy leaf by tearing the leaf parallel to the mid-vein. **In the dark**, peel the lower epidermis (guard cells are in the epidermal layer of cells) from the leaf using forceps. DO **NOT** ALLOW THE EPIDERMAL STRIP TO **DRY**. Place several strips into the solution.

3. Place plates under light for **1 hour**, except for 'dark' treatment – put that one in a drawer.

4. Examine the light and dark treated sections under the microscope. Look for pairs of guard cells in a given area. Examine at least 20 pairs for each treatment. Note the number of stomates that are wide open, open, nearly closed or closed.

Present results in a table:

Treatment	**Number of stomata**			
	Wide Open	Open	Nearly closed	Closed
Dark, KCl				
Light, KCl				
Light, Mannitol				
Light, Choline Cl				
Light, ABA, KCl				

Data Analyses and interpretation: *Note your observations and propose a model explaining one or more reasons for what you saw.* ***Include this in your lab report!***

5. One student will present each group's results to the class (5 min). The entire class will discuss the findings and reach conclusions.

Questions:

i) Under what environmental conditions does ABA level increase in plants?
(Hint: see Taiz and Zeiger, Ch. 23)

ii) Why would light cause a change in stomatal aperture? Hint- When does a plant sense changes in light and dark in the natural environment?

iii). What specific molecular events cause the opening of the stomatal aperture? What specific changes occurred in the guard cells?

iv). Why is there a difference in your result when you used mannitol instead of KCl?

References:

Taiz and Zeiger, 2010. Ch. 3, 4; Water potential
Taiz and Zeiger, 2010. Ch. 18, Guard cell movement
Taiz and Zeiger, 2010. Ch. 23, ABA and water deficit