## Investigating the effect of carbon dioxide concentration on stomatal density

Tiny pores in the epidermis of leaves called **stomata** control the movement of gases into and out of a leaf. They are an important part of maintaining homeostasis in plants.

Environmental factors, such as availability of water, carbon dioxide concentration, and temperature, can determine the stomatal density of a plant.

In this investigation, three plants of the same species will be incubated for six weeks in different concentrations of carbon dioxide.

The stomatal density will be measured before and after the incubation period using an epidermal peel, to observe the effect of carbon dioxide concentration.

A leaf of approximately the same size is taken from each of the three plants and the average stomatal density of each leaf is measured.

One plant is kept in a high concentration of carbon dioxide, the other in a low concentration, and the third plant is kept in atmospheric levels.

A high concentration of carbon dioxide is created and maintained by dripping hydrochloric acid onto marble chips every 2–3 days to release carbon dioxide.

A low concentration is created and maintained by the presence of soda lime, which removes carbon dioxide from the air.

The plants are left for six weeks and they are watered with tap water once every 2-3 days.

After six weeks, new leaves should have grown. A young leaf of roughly the same size is picked from each plant.

The stomatal density is the number of stomata per milimeter squared. This can be found by viewing an epidermal peel with a microscope. The area of the field of view is calculated and the number of stomata counted.

To check that each leaf is approximately the same size, and therefore age, each leaf is placed on a piece of graph paper and its outline is drawn with a sharp pencil.

The area is estimated by counting the number of squares within the outline.

Each leaf is torn along a vein in the direction from apex to the petiole.

The epidermis should be visible as a transparent film that has separated from the rest of the leaf.

A section of epidermis is removed.

Each epidermal peel is placed into a different watch glass and eosin stain is added. This is left for at least 2 minutes to penetrate the plant cells.

The peels are transferred to distilled water to remove excess stain.

Each peel is then transferred to a labelled microscope slide.

A drop of glycerol is added, and a cover slip is placed carefully on top to avoid trapping air bubbles.

The slides are now ready for viewing under a microscope.

At 100 times magnification, stomata should be obvious as small pores surrounded by guard cells.

The visible area of the leaf is calculated, and the number of stomata is recorded.

The slide is moved so that another area is visible. The area is calculated again, and the number of stomata is counted. This is repeated for five random areas so that a mean can be calculated.

This whole process is carried out for each leaf. A suitable graph of the results is plotted and a statistical test is used to determine if any observed difference in stomatal density is significant.

The data can be used to make conclusions about the relationship between carbon dioxide concentration in the atmosphere and plant responses to environmental conditions.

This relationship has implications for the commercial production of crops.

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