

## Topic 1 – Practical 1

### *Stages of mitosis in a root tip*

#### Safety

- Care must be taken when handling acetic alcohol, hydrochloric acid and Feulgen stain.
- Eye protection and laboratory aprons should be worn.
- If chemicals come in contact with skin or eyes, rinse with plenty of water.

#### Apparatus and materials

- garlic bulb (or onion or beans)
- boiling tubes
- three test tubes with stoppers
- two Petri dishes
- fine forceps
- water bath at 60°C
- 1 mol dm<sup>-3</sup> hydrochloric acid (5 cm<sup>3</sup>)
- Feulgen stain
- distilled water
- acetic alcohol (10 cm<sup>3</sup>)
- mounted needles
- slides and coverslips
- tissue or blotting paper
- microscope

#### Introduction

Chromosomes can be observed in dividing cells found in the root tips of garlic or onion ( $2n = 16$ ) or the broad bean ( $2n = 12$ ). The tip of a root contains meristem tissue where there are cells dividing by mitosis. The three-dimensional structure of the meristem must be disrupted and a single layer of cells separated out. The middle lamella holding cells together can be dissolved by hydrochloric acid, which does not damage the cellulose cell wall. The nuclei of the cells are stained with dye that does not stain the cytoplasm.

#### Procedure

- 1 To grow the roots, suspend a bulb of garlic in the mouth of a boiling tube full of water for about 4 days. Avoid disturbance, which may affect cell division. (The roots may have been grown for you.)
- 2 When the roots are about 2 cm long remove 1 cm from the end.
- 3 Fix the root material in a closed test tube of acetic alcohol overnight.
- 4 Remove the root tips using fine forceps and wash in distilled water in a Petri dish.
- 5 Place the root tips in a test tube and cover with 1 mol dm<sup>-3</sup> hydrochloric acid for about five minutes (slightly longer for bean roots) in a water bath at 60°C. This treatment removes the middle lamellae and hydrolyses DNA.
- 6 Transfer the root tips to a Petri dish and wash with distilled water.
- 7 Transfer the root tips to a test tube containing Feulgen stain. Seal the tube keep cool for about three hours.

**NB** *If preferred these preparatory stages can be carried out before class by a technician and students can complete the following stages.*

- 8 Remove a root tip and place on a microscope slide in a drop of acetic alcohol.
- 9 Cut off and keep the end 1–2 mm of root, removing the remainder from the slide.
- 10 Use mounted needles to tease the material apart, and then cover with a coverslip.
- 11 On a flat surface, cover with several layers of tissue paper and press firmly, do not allow the coverslip to move across the slide.

The slide can then be examined under a microscope on low and high power to observe the stages of mitosis.

- 1 Make careful drawings of your observations, below. Show all the stages of mitosis to the same magnification. Do not show unnecessary detail.
  - Use a sharpened pencil (HB or H) to draw single clear lines.
  - Do not leave gaps or sketch as you draw the edges of cells, but use clear, continuous lines.
  - Use a ruler and straight lines to position labels at the side of your drawings for structures you observe.
  - Give your drawings a title and show what magnification was used when you observed the cells.

- 2** The length of a cell in a diagram is 20 mm; the actual size of the cell it represents is 2  $\mu\text{m}$ . Calculate the magnification of the diagram.