

## Expt: 5 Study of mitosis in onion root tips

### Aim:

To prepare a temporary mount of onion root tip to study mitosis.

### Materials Required:

Onion, Watch glass, Glass slide, Blotting paper, Aceto-alcohol, Coverslip, Water, Hydrochloric acid, Acetocarmine Stain, Burner, Forceps, Dropper, Blade, Needle, Compound microscope.

### Procedure:

Take one or two preserved roots, wash them in water on a clean and grease free slide.

Add a drop of HCl on the root tip followed by 2–3 drops of acetocarmine stain on it.

Leave the slide for 5-10 minutes on a hot plate or warm it slightly on spirit lamp.

Care should be taken that the stain is not dried up.

Blot the excess stain using blotting paper.

Cut the deeply stained (2–3 mm) portion of the root tip.

Place it on the slide and discard the remaining portion of root.

Add one or two drops of water on the root tip after (10–20 seconds) and place a cover slip on it avoiding air bubbles.

Tap the cover slip gently using the blunt end of a pencil so that the meristematic tissue of the root tip below the cover slip is properly squashed and spread as a thin layer of cells.

This preparation of onion root tip cells is ready for the study of mitosis.

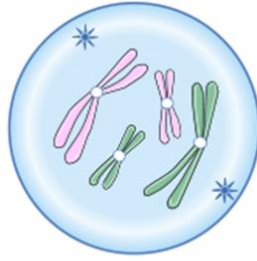
## Stages of Mitosis

### Prophase:

Condensation of chromosomes occur.

Chromosomes consist of **two chromatids** attached together at the centromere.

Nuclear membrane and nucleolus disappear.



Prophase

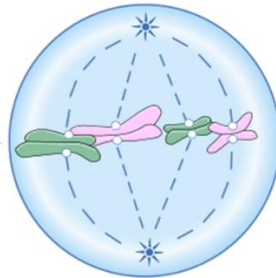
### **Metaphase:**

Condensation of chromosomes is completed and they can be observed clearly under the microscope.

Small **disc-shaped structures** found at the surface of the centromeres are called **kinetochores**.

Chromosomes are arranged at the equator or centre.

Spindle fibres get attached at the kinetochores.



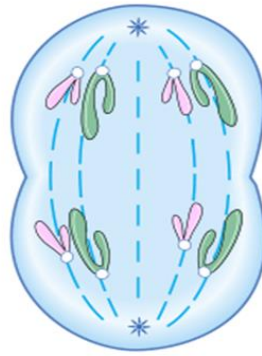
Metaphase

### **Anaphase:**

The spindle fibres contract.

Centromeres split and chromatids move towards the opposite poles.

The chromosomes may look like the shape of alphabets 'V', 'J' or 'I' depending upon the position of centromere in them.



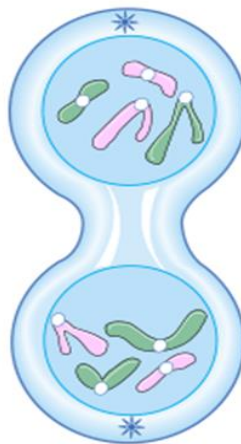
Anaphase

**Telophase:**

Chromosomes have reached the opposite poles. They decondense and lose their structure.

The chromatin material tends to collect in a mass in the two poles.

Nuclear membrane, Nucleolus, Golgi complex and ER reappear.



Telophase

**Cytokinesis:**

In animal cells, cytokinesis occurs by the appearance of a furrow in the plasma membrane.

The furrow gradually deepens and ultimately joins in the centre dividing the cell cytoplasm into two.

In plant cells, wall formation starts at the centre of the cell and grows outward to meet the existing lateral walls.



## Growing of onion root tips

Select a few medium-sized onion bulbs.

Remove carefully the dry roots present.

Regrow root tips by placing the bulbs on glass tubes (of about 3–4 cm. diameter) filled with water.

Care should be taken so that the stem portion of the bulb (basal part) just touches the water.

A few drops of water may be added periodically to compensate evaporation losses.

It may take 3–6 days to grow.

Cut 2–3 cm long freshly grown roots and transfer them to fixative, i.e., aceto-alcohol (1:3 glacial acetic acid : ethanol).

Keep the root tips in the fixative for 24 hours and then transfer them to 70% ethanol (for preservation and use in future).

Onion root-tip cells have a cell cycle of approximately 24-hour duration.

They divide once in 24 hours, and this division usually takes place about two hours after sunrise.

Therefore, roots grown on water should be cut only at that time to score maximum number of dividing cells.

\*\*\*\*\*