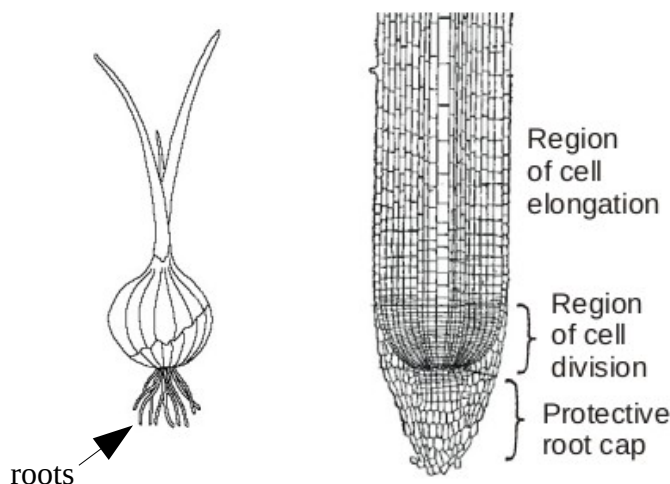


Aim: Preparation of temporary stained squash of onion root tip to study various stages of mitosis

Principle: The genetic information of organisms is located in **DNA molecules** organized in the form of **chromosomes**. Each human cell for example has 46 chromosomes, while each cell of onion has 8 chromosomes. All cells replicate their DNA before dividing. During DNA replication, the two DNA strands separate, and for each original strand a new complimentary strand is produced, giving rise to two identical DNA molecules. Each pair of identical DNA molecules (called **sister chromatids**) remain attached to each other at a region called the **centromere**. DNA replication in eukaryotes is followed by a process called **mitosis** which ensures that each daughter cell receives one copy of each one of the replicated chromosomes. Thus mitosis is a kind of cell division where each dividing cell gives rise to two daughter cells. The number of chromosomes remains the same before and after mitosis, but each chromosome has only one chromatid after completion of mitosis, whereas it has two chromatids while starting mitosis, hence the DNA content after mitosis is half of that when mitosis starts.

During mitosis the chromosomes pass through several stages – **prophase, metaphase, anaphase** and **telophase**. The division of cytoplasm is called **cytokinesis** and occurs during teophase. During early prophase, the chromosomes supercoil and the spindle fibres begin to form between centrosomes located at the pole of the cells. The nuclear membrane disintegrates at this time. During late prophase the spindle fibres attach to the centromere of each pair of sister chromatids and they begin to move toward the center of the cell. During metaphase the chromosomes rest along the center plane of the cell. During anaphase, the centromeres split and the sister chromatids begin to migrate toward the opposite poles of the cell. During telophase, the chromosomes at either end of the cell begin to cluster together, and the formation of new nuclear membrane starts. Cytokinesis also occurs during telophase, giving rise to two separate daughter cells. Cell plate appears between the two daughter cells – this is the new cell wall forming between the two cells.

Mitosis can be observed from onion (*Allium cepa*) root tips. The roots are easy to grow in large numbers and can be grown by keeping the root region of an onion immersed in water for a few days. The cells at the tip of the root are actively dividing, hence many cells will be in stages of mitosis. The chromosomes can be stained to make them easily observable, and the tips can be prepared in a way that allows them to be flattened or squashed on a slide so that chromosomes of individual cells can be observed. The extreme end of each root has a root cap that has cells that cover and protect the underlying growing region as the root grows and is pushed through the soil. Immediately after the root cap is a region of cell division where cells are actively dividing but not growing in size. After this zone of cell division, starts the region of cell elongation, where cells are increasing in size but not dividing. Mitosis is best observed from the region of cell division.



Requirements: growing onion roots, razor blade, test tube, watch glass, forceps, brush, water, N/10 HCl, water bath, thermometer, aceto-orcein stain, 45% acetic acid, blotting paper, slide, cover slip, rubber-ended pencil, nail polish, microscope.

Procedure:

1. About 1 cm long root tips were cut from the distal ends of onion roots and placed in N/10 HCl in a test tube.
2. The root tips were incubated with N/10 HCl in a 60°C water bath for 10-15 minutes (this softens the tissue making it easier to spread during squashing).
3. HCl was discarded and the root tips were washed with water.
4. A root tip was placed on a glass slide and a few drops of aceto-orcein stain was added and allowed to stain for 15-20 minutes.
5. Stain was removed by soaking in blotting paper (or tissue paper), and 45% acetic acid was added on the root tip tissue and left for 2 minutes.
6. Cover slip was placed on the stained root tip and the root tip was squashed with the rubber-end of a rubber-ended pencil, keeping the cover slip covered under the folds of a blotting paper (to soak the liquid released from below the cover slip during squashing). Care should be taken while squashing so that the cover slip does not rotate or move sideways otherwise the spread of the cells will not be good.
7. The edges of the cover slip were sealed by nail polish.
8. The slide was observed under a microscope to observe different stages of mitosis.

Observation:

Interphase – Nucleus appeared more or less uniformly stained and chromatin or chromosomes were not clearly visible. Nuclear membrane was present.

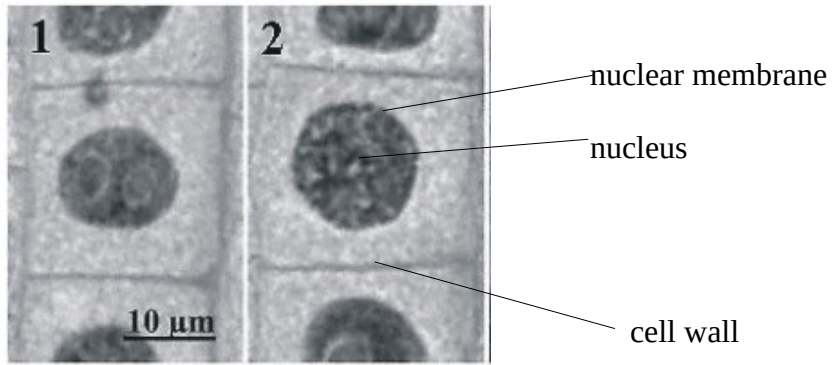
Prophase – Chromosomes were visible inside the nucleus and the nuclear membrane was present.

Metaphase – Chromosomes appeared as thicker (more densely condensed) than in prophase, and were arranged along the central portion of the cell.

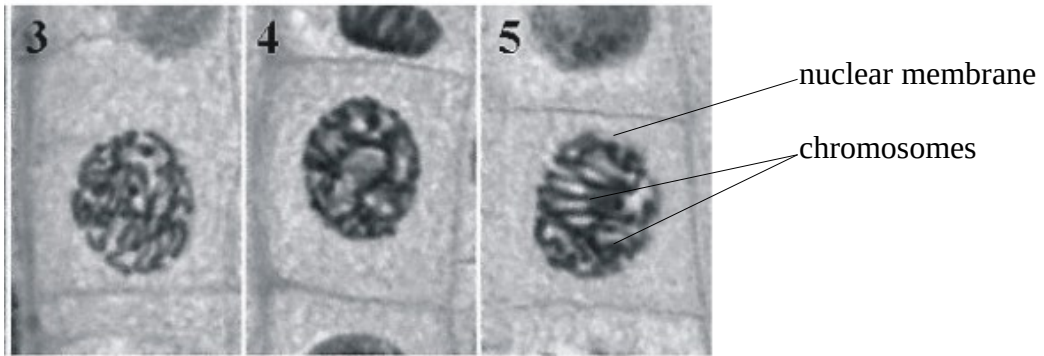
Anaphase – Chromosomes were divided into two groups which appeared to be moving away from each other towards the opposite ends of the cell.

Telophase – Chromosomes were decondensed, forming chromatin, and the nuclear membranes were partly or fully present. Cell wall was also present between the two daughter cells. The length of each daughter cell was approximately half of the cell observed in the preceding earlier stages.

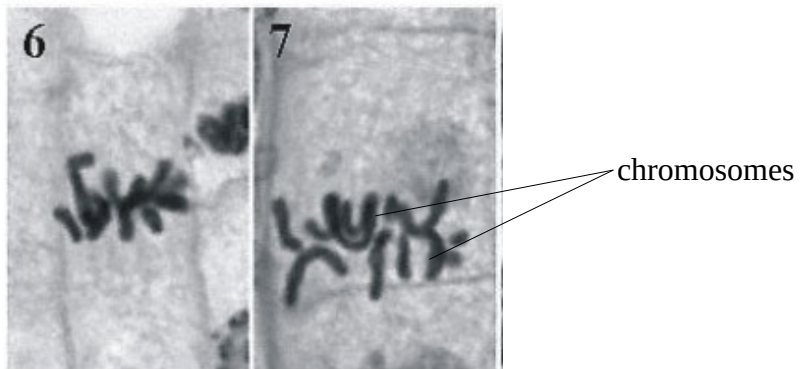
Interphase:



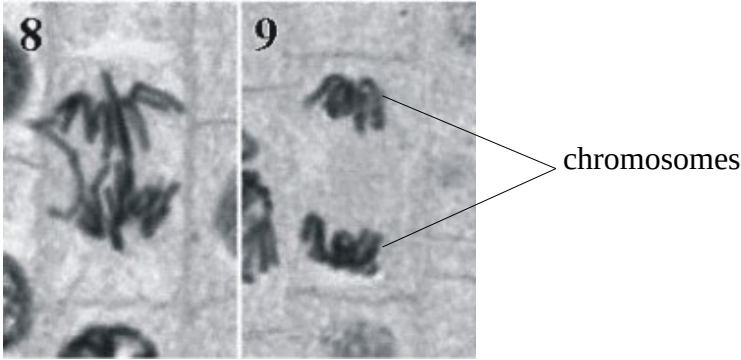
Prophase:



Metaphase:



Anaphase:



Telophase:

