

**AIM :** Study of different stages of Mitosis from temporary acetocarmine squash preparation of root-tip cells of onion (*Allium cepa*).

## **INTRODUCTION:**

The reason for using onion root tips for viewing mitosis –

The roots are easy to grow in large numbers, the cells at the tip of the roots are highly dividing and thus many cells will be in stages of mitosis, the chromosomes can be stained to make them more easily observable.

There are three cellular regions near the tip of an onion tip –

- i. The **root cap** contains cells that cover and protect the underlying growth region.
- ii. The **region of cell division** (or meristem), where cells are actively dividing but not increasing significantly in size.
- iii. In the **region of cell elongation**, cells are increasing in size but not dividing.

## **REQUIREMENTS:**

### **CHEMICALS:**

1. 0.1 N HCl (1ml conc. HCl added in 119ml Distilled water).
2. 70 % and 90 % ethanol.
3. Acetic-alcohol fixative (1: 3 acetomethanol fixative).  
Glacial acetic acid = 1 part  
Ethyl/ methyl alcohol = 3 parts
4. 2 % acetocarmine stain
5. 45 % acetic acid.

### **MATERIALS:**

Slide, Cover slip, Watch glass, squashing needle, spirit lamp, blotting paper, Onion root tips.

## **PROCEDURE:**

### ***Fixation of Root tips :***

1. Freshly cut onion root tips (1 cm long) are fixed in acetometanol fixative for overnight in a specimen tube.
2. Then, 90 % ethanol is added after decanting the fixative. Finally 70 % ethanol added after decanting.

The root tips can be stored in 70 % ethanol for a long period of time in tightly closed container at 40 °C.

***Staining and making squash preparation:***

1. Root tips are treated with 0.1%N HCl for 1 min. This will soften the cell wall.
2. Root tips are rinsed in water and transferred to acetocarmine stain for 30 mins.
3. A drop of 45 % acetic acid is taken on a slide having one root tip and left for 1-2 min. If acetic acid drop becomes coloured, it is decanted and a fresh drop is added.
4. A cover glass is placed on the root tip and squashed it using a rubber-end pencil under the folds of a blotting paper.
5. Then edges of the cover glass is sealed with molten wax or with nail polish immediately to prevent drying of acetic acid film and entry of air bubbles.
6. Now the slide is observed under microscope.

**OBSERVATION:**

The following stages of mitosis are observed under microscope.

**Interphase :** Interphase is also called the resting phase. It is the longest phase.

1. Replication of DNA takes place during this phase.
2. The chromosomes are thinly coiled.
3. Presence of nucleolus and nuclear membrane.

**Prophase :**

1. The chromatin appears as a network of fine threads. If the cell is in early stage of prophase then the chromatin fibres (chromosomes) are very thin, while the cells at late prophase show comparatively thicker chromatin fibres.
2. Spindle formation is initiated.
3. The nuclear membrane and nucleolus start disappearing at the later stage.

**Metaphase :**

1. Disappearance of the nucleolus and nuclear membrane.
2. Chromosomes are at their maximum condensed state with two chromatids joined at their centromere.
3. Spindle formation complete.
4. The chromosomes align in the equatorial position of the spindle and form the equatorial plate that is at right angle to the spindle axis.
5. The centromeres are arranged exactly at the equatorial plate.

**Anaphase :**

1. The centromere of the chromosomes divides and the two chromatids of each pair separate.
2. Each chromatid now represents a separate chromosome and it starts moving towards the opposite poles.
3. The daughter chromosomes assume 'V' or 'J' shapes or depending upon the position of centromere in them.

### Telophase :

1. Chromosomes reach the opposite poles and look like a mass of chromatin.
2. The new nuclear membrane starts to reappear around each set of chromosomes.
3. The nucleolus gets reorganized.

### Cytokinesis :

In plants a cell plate is formed in the middle after telophase, finally dividing the cells into two.

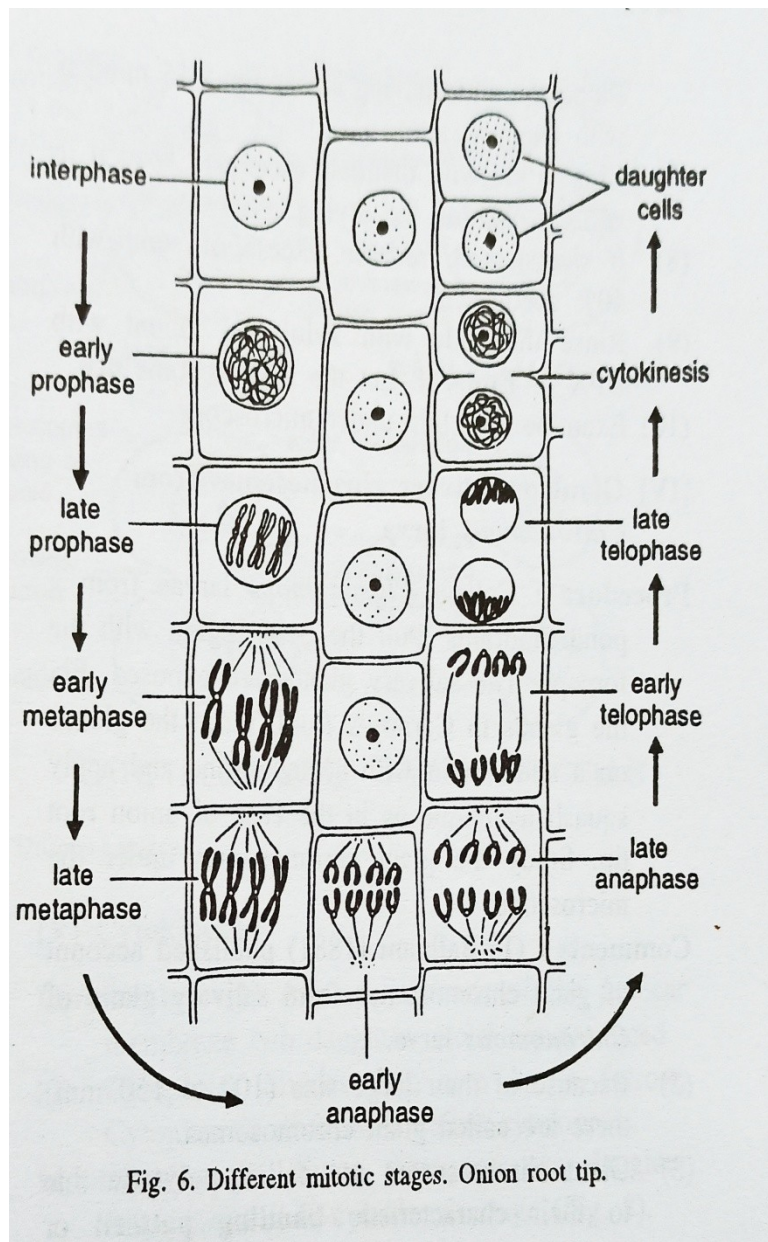
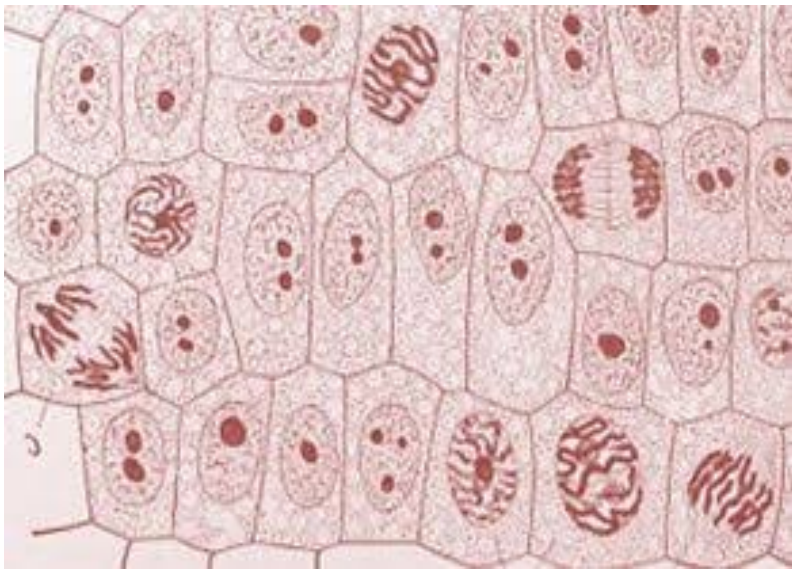
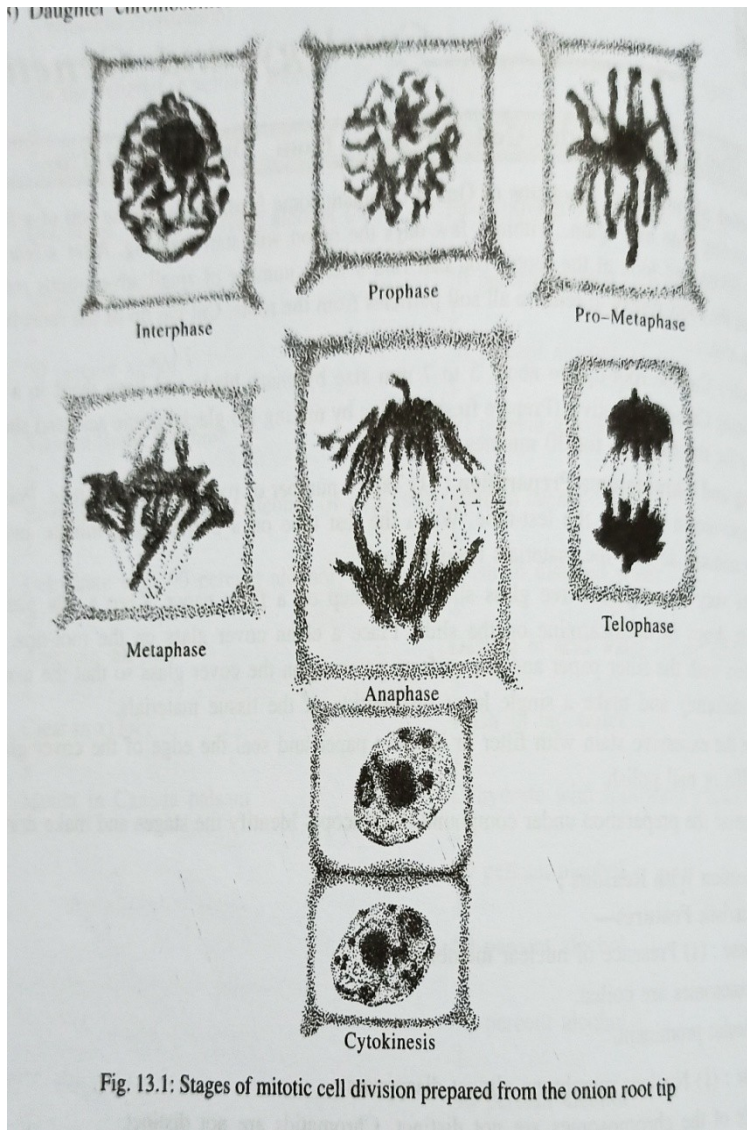
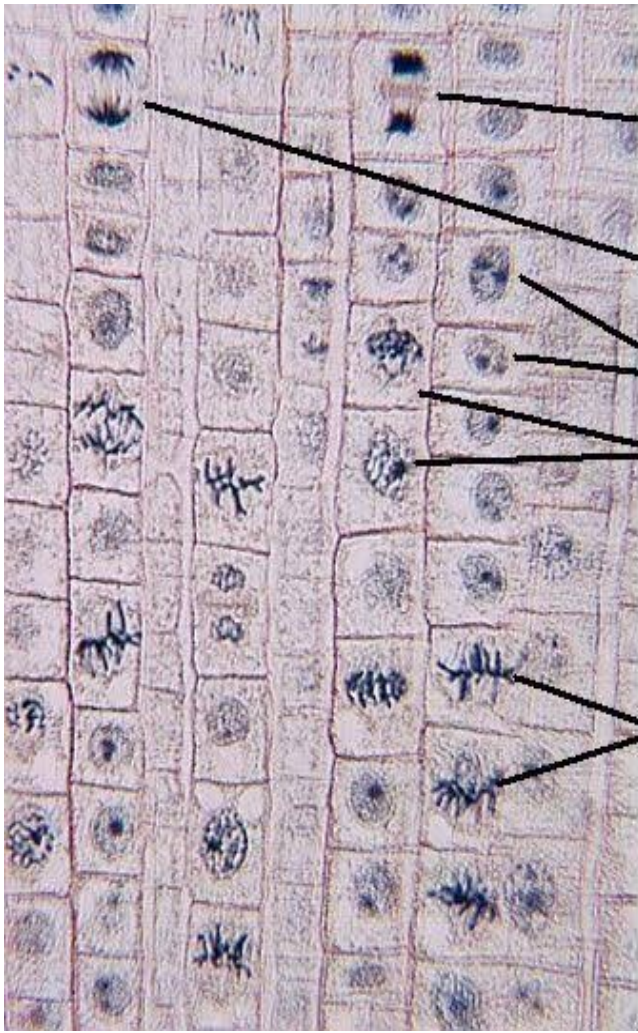


Fig. 6. Different mitotic stages. Onion root tip.

Diagram for practical copy





Telophase

Anaphase

Interphase

Prophase

Metaphase

