

SQUASH PREPARATION OF ONION ROOT TIP FOR MITOTIC STAGES

Aim

To understand the process and different stages of mitosis and to visualize different phases of mitosis.

Principle

The genetic information of all organisms resides in the individual DNA molecules or chromosomes. An onion cell possesses eight chromosomes whereas human cells possess forty six chromosomes.

In 1842, C. Nägeli first saw chromosomes and in 1888 W. Waldeyer named them. Walther Flemming studied and named the process of cell division as mitosis. Cell division occurs rapidly in growing root tips of sprouting seeds or bulbs.

An onion root tip is a rapidly growing part of the onion and thus many cells will be in different stages of mitosis. The onion root tips can be prepared and squashed in a way that allows them to be flattened on a microscopic slide, so that the chromosomes of individual cells can be observed easily. The super coiled chromosomes during different stages of mitosis present in the onion root tip cells can be visualized by treating with DNA specific stains, like Feulgen stain and Acetocarmine stain.

Materials required

Onion plant with root, acetocarmine stain, 1 N HCl, Scissors, Forceps, Razor blade, Microscopic slides and cover slips, Water bath, Light Microscope.

Procedure

1. Cut the tip 5 to 8 mm from the tip of the freshly sprouted root. Discard the rest of the root.
2. Wash them in water on a clean microscope slide.
3. Place one drop of 1N HCL on the root tip and add 2-3 drops of acetocarmine stain to the slide.
4. Warm the slide gently over the alcohol lamp for about one minute. (Do not allow the slide to get hot to the touch; you don't want to cook either your fingers or the root. Do not let the root dry out).
5. Carefully blot the excess stain with a blotting paper.
6. After (10 to 20 seconds) put one or two drops of water and blot them carefully using blotting paper.
7. Again put a drop of water on the root tip and mount a cover slip on it avoiding air bubbles.
8. Squash the slide with your thumb using a firm and even pressure. (Avoid squashing with such force that the cover slip breaks or slides).
9. Observe it under a compound microscope in 10x objective. Scan and narrow down to a region containing dividing cells and switch to 40x for a better view.

The process of Mitosis is divided into four stages: Prophase, Metaphase, Anaphase and Telophase.

Prophase: During this stage, the chromosomes super coil, condense and become visible for first time during the cell cycle. The spindle fibers start forming. The nuclear membrane starts disintegrating.

Metaphase: During this stage, the spindle fibers reach and attach to centromere of each sister chromatids. The chromosomes align along the center plane of the cell. The nuclear membrane disintegrates completely.

Anaphase: During this stage, the centromeres start splitting and the sister chromatids begin to migrating towards the opposite poles of the cell.

Telophase: During this stage, the chromosomes are clustered on the either end of the cell. The nuclear membrane starts reforming. The cell plate (new cell wall) starts to form between the two daughter nuclei. This will be followed by cytokinesis.

