

Experiment no. 5: Practice on mitotic cell division

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

PRINCIPLE:

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.

MATERIAL REQUIRED:

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| 1. Onion root tip | 2. Acetoorcein (2%) | 3. 1 N HCl |
| 4. Scissors | 5. Forceps | 6. Razor blade |
| 7. Dropper | 8. Vial | 9. Dissection probe with wooden back |
| 10. Light Microscope | 11. 45% acetic acid sol. | 12. Microscopic slides and cover slips |

PROCEDURE:

- ❖ Present using a sharp blade.
- ❖ Grow root tips by placing the bulbs in a beaker filled with water.
- ❖ New roots may take 3–6 days to grow.
- ❖ Cut off 2–3 cm of freshly grown roots and let them drop into a watch glass.
- ❖ Using forceps transfer them to the vial containing freshly prepared fixative of aceto-alcohol (1:3: glacial acetic acid: ethanol).
- ❖ Keep the root tips in the fixative for 24 hours.
- ❖ Using forceps take one root and place it on a clean glass slide.
- ❖ Using a dropper, place one drop of 1N HCl on the root tip followed by 2–3 drops of Acetoorcein stain.
- ❖ Warm it slightly on burner. Care should be taken that the stain is not dried up.
- ❖ Carefully blot the excess stain using filter paper.
- ❖ Using a blade, cut the comparatively more stained tip portion of the root, retains it on the slide and discard the remaining portion.
- ❖ After that, put one drop of 45% acetic acid sol on the root tip.
- ❖ Mount a cover slip on it using a needle. Care should be taken to avoid bubble formation.
- ❖ Now, slowly tap the cover slip using the blunt end of a needle 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 now ready for the study of mitosis.
- ❖ Place the slide under the compound microscope and observe the different stages of mitosis.

- ❖ Various stages of mitosis are prophase, metaphase, anaphase and telophase can be observed.

PROCEDURE:

Growing of root tips

Select a few medium-sized onion bulbs. Carefully remove the dry roots present. Grow 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. New roots may take 3–6 days to grow. Cut 2–3 cm long freshly grown roots and transfer them to freshly prepared 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, i.e., 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.

STUDY OF CELL:

Place the slide on the stage of a good quality compound microscope. First observe it under the lower magnification (10 X objective) to search for the area having a few dividing cells. Examine the dividing cells under higher magnification of the microscope to observe the detailed features of mitosis.

OBSERVATION:

The stages of mitosis can be broadly categorized into two parts: karyokinesis (division of nucleus) followed by cytokinesis (division of cytoplasm, and ultimately of the cell). Those cells, which are not in the phases of cell division, are considered to be in interphase. You may observe that most of the cells in a microscope field are in interphase

INTERPHASE

The cells are mostly rectangular, oval or even circular in shape, with almost centrally situated densely stained nucleus. The chromatic (colored) material of the nucleus is homogeneous and looks granular. The boundary of the nucleus is distinct. One or few nucleoli (sing: nucleolus) can also be observed inside the nucleus

DISCUSSION

Mitotic index (MI) is defined as a ratio of the total number of dividing cells (n) and the total number of cells (N) in a particular focus chosen randomly under the microscope and is calculated as $MI = n/N \times 100$ By randomly selecting 5 to 10 such foci, one can estimate the mitotic index for a given type.

The effect of different samples of water (polluted or contaminated) can be assayed on the mitotic-index (an indicative feature of somatic growth rate in them). Further, the impacts of different types of pollutants on different phases of mitosis can also be assayed.

Fig.: Different stages of mitosis

