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Swotting the Behaviour of Chromosomes at Mitotic Cell Division in the Root Tip of Onion

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ABSTRACT

As a part of the course, PBG 112 – Principles of Genetics and Cytogenetics (2+1), by the students of B.Sc., (Hons.) Horticulture (2023 Batch) at Adhiparasakthi Horticultural College, the study was undertaken on the behavior of chromosomes at different phases in mitosis. In this context, we have observed the three different phases of mitosis viz., Prophase, Anaphase, and Telophase. For which, the onion root tip was used as an experimental material for studying the behavior of chromosomes at different phases in Mitotic cell division. All three phases of mitosis were observed at 40 X magnification.

INTRODUCTION

The ability of all living cells to proliferate and divide is one of their most crucial traits. The process by which a cell duplicates itself for an organism's growth and reproduction is known as

cell division. The process by which cellular components are distributed equally among daughter cells are intricate. Replicated DNA splits into two daughter nuclei during the mitotic phase without undergoing recombination. The two main events of the mitotic phase are the division of the nucleus (Karyokinesis) and the division of the cytoplasm (Cytokinesis).

MITOTIC PHASES

Interphase: It is the time between subsequent cell divisions, during which processes related to growth and mitotic preparation take place. It consists of the pre-DNA replication phase, or the "G1" phase (Gap 1 phase). This is where the RNA and protein synthesis that is necessary for DNA replication occurs. The synthesis phase, or "S" phase, is the time during interphase when DNA is synthesized. "G2" phase is the phase that follows DNA replication.

Prophase: Chromosomes undergo coiling and condensation, which gives them a structure resembling a thread. The two identical longitudinal splits, known as sister chromatids or identical chromosomes, and each chromosome is joined by a common centromere. centriole migration to the cell's opposite ends. The Formation of spindle fibers begins. Nuclear membrane disintegrates and nucleolus disappears.

Metaphase: Spindle fiber formation is finished. At the centromere, chromosomes are joined to the spindle fibers. Every chromosome moves and positions on the equatorial plate during metaphase. Each chromosome's sister chromatids are connected at the centromere, but their arms is free. Chromosomes can be counted and are easily observable.

Anaphase: This is the shortest stage of the division of mitosis. The centromere divides in two as the spindle fibers start to contract. Chromatid segregation occurs when sister chromatids split off and travel to opposing poles. Daughter or new chromosomes are the terms used to describe the split sister chromatids. Depending on where the centromere is located, the arms of each chromosome pull behind it to give it a unique shape. (Rod, L, J, and V-shaped).

Telophase: The daughter chromosomes are now at the poles that face each other. The spindle fibers start to break down. The nuclear membrane is restored. The nucleolus undergoes reform. Once more, as chromosomes uncoil and unfold, they get longer and thinner.

Cytokinesis: It is the cytoplasm's division. This phase typically precedes the G1 phase of interphase and comes after telophase. Animals undergo cytokinesis by creating a cleavage furrow that pinches and deepens the cell into two daughter cells. When a plant undergoes cytokinesis, a cell plate forms in the center of the cell and spreads laterally throughout the cell. The cell plate is then supplemented with cellulose and strengthening agents to create a new cell wall.

PREPARATION OF FIXATIVES

The word "killing" in cytology refers to the abrupt end of a tissue's individual cells' life cycle. The main agent that kills is alcohol. Cells or tissues are abruptly killed during the killing and fixing process, leaving the chemical makeup and morphological structure of the cells largely

unchanged. Despite being separate processes, both are typically achieved using a single fluid, which is typically a blend of compatible chemical reagents.

Carnoy's Fluid I (Glacial Acetic Acid – 1 Part; Absolute Ethanol – 3 Part). It works well for mending all materials made of plants, animals, and people. Fixation times range from 15 minutes to 24 hours. The specimen should be cleaned in 95% ethanol with two or three times after fixation in order to get rid of any acetic acid that might have interfered with the staining process. The components of Carnoy's fluid I and Farmer's solution are identical.

PREPARATION OF STAINS

Staining is the process of coloring the cells using specific inorganic or organic dyes. The choice of dye or stain for a given material is determined by the substance's chemical makeup, the fixative's pH level, and the stain's chemical reactivity with the material. The majority of cytological stains are dye solutions made of aromatic organic compounds with chromophoric and auxochromic groups as their active chemical groups. The auxochromic group allows the dye to stick to the tissue or material, while the chromophoric group gives the dye its color.

Acetocarmine: Carmine stain is dissolved in acetic acid to create acetocarmine stain. Gently boil 45 milliliters of glacial acetic acid and 55 milliliters of distilled water to obtain 45 percent acetic acid. After bringing 45% acetic acid to a boil, 1g of carmine powder is added and the mixture is left to boil for a few minutes. The solution is taken off the burner and allowed to cool to room temperature after it has boiled. After that, the mixture is filtered through Whatman No. 1 filter paper and placed in a clear bottle. The filtrate has a color of light red. For deep staining and preservation, ferric acetate and chloride may be added as needed.

PREPARATION OF ROOT TIP SQUASHES FOR MITOSIS STUDY

PRETREATMENT

The roots are carefully cut off, cleaned of dirt, and submerged in a chemical known as para-dichlorobenzene for pretreating. Depending on the material, the pretreatment process can take anywhere from one to two hours. Forty minutes is plenty of time for onion root tips. The material can be stored in the refrigerator following pretreatment.

WASHING AND FIXING

Since the presence of fluid, even in minute amounts, negatively impacts the staining process, root tips are extracted from the fluid and thoroughly cleaned with distilled water for five, six, or even more times. After that, the roots are fixed for an hour in 3:1 aceto-alcohol (Carnoy's fluid I). Following this, the root tips can be immediately used after being immersed in 70% alcohol.

HYDROLYZING

Five to six root tips are removed, cleaned with distilled water, and any remaining moisture is blotted off with blotting paper. After transferring the root tips to a watch glass, a few drops of diluted 1N HCl are added to hydrolyze them (1N HCl is made by mixing one part of concentrated HCl with eleven parts of distilled water). To get rid of any remaining HCl, the root tips need to be thoroughly cleaned after softening.

STAINING

The hydrolyzed onion root tips are placed on a slide, firmly compressed, and stained with 1% aceto-orcein in accordance with the instructions provided below. They are then examined under a light microscope.

PROTOCOL

1. Excise 5 to 7 root tip of onion and pretreat with Para dichloro benzene.
2. Fix the root tip on fixative (Carnoy's Fluid I) for 6 – 8 hours.
3. Hydrolyze the root tip for 10 mins in 1N Hydrochloric acid.
4. Wash the hydrolyzed root tips in distilled water 3 times.
5. Remove the excess water from the root tip using a blotting sheet.
6. Cut off 1mm of the root tip placed on the glass slides and add 1 drop of 1% Acetocarmine.
7. Place the cover slip on the root tip and give uniform pressure using your thumb through blotting paper.
8. Heat the glass slide with a flame for deep staining.
9. Examine the slides under Microscope (4X, 10X, 40X, 100X).
10. Record the Observation.

OBSERVATIONS

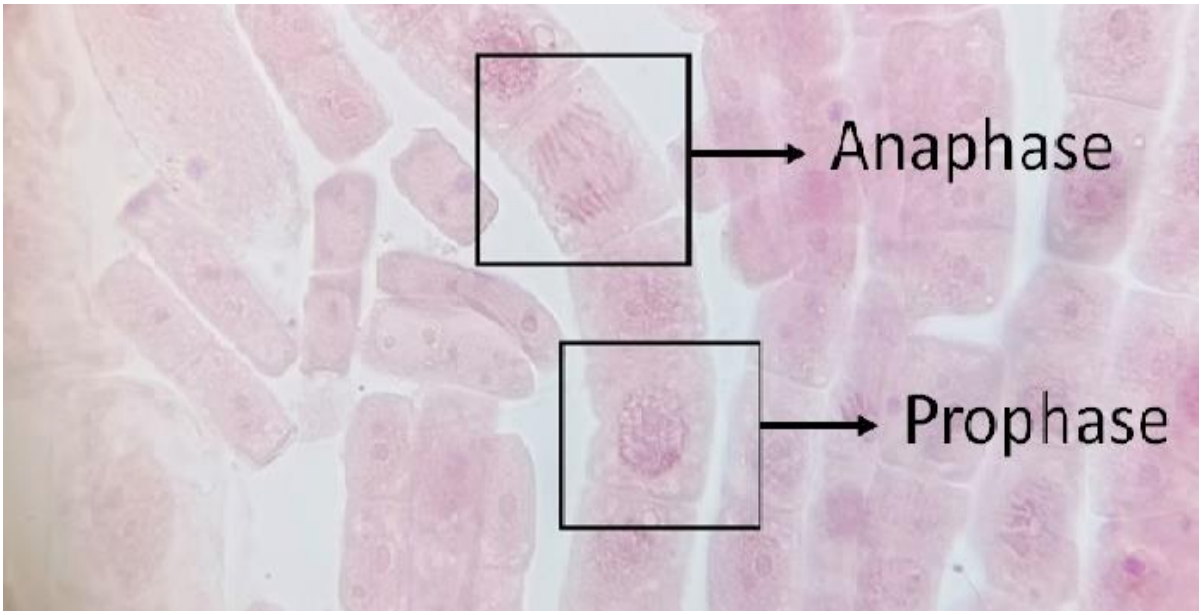

Figure 1. Cell Cycle (Anaphase and Prophase)

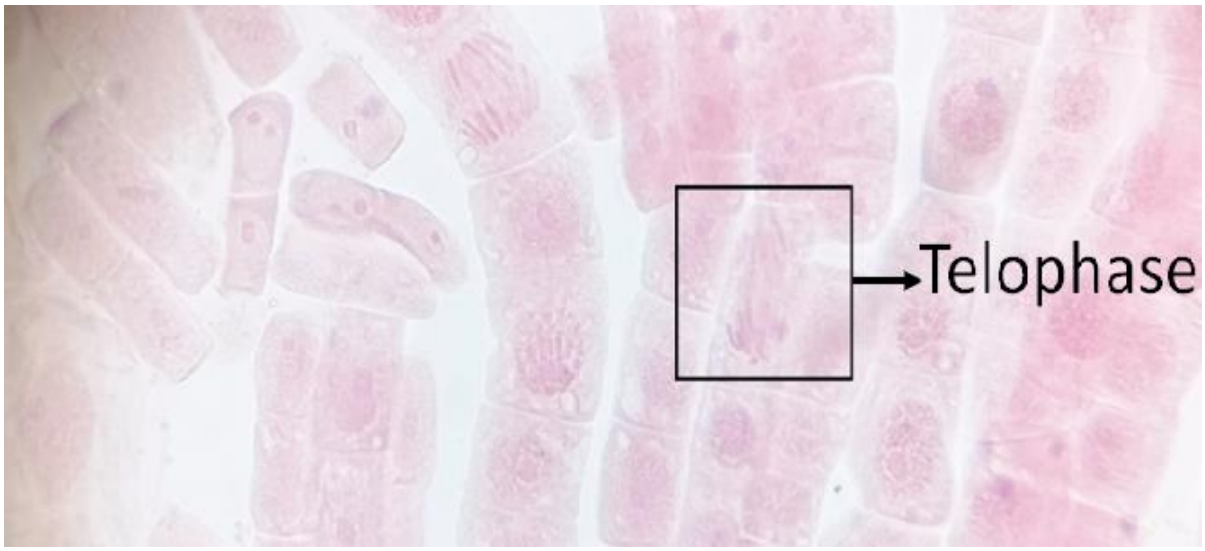

Figure 2. Cell Cycle (Telophase)

CONCLUSION

As per the above protocol we have used the onion root tip as a genetic material for the study of different mitotic phases. In that, we have observed the different phases viz., Prophase, Anaphase, Telophase at 40 X magnification. The image have been captured and given in Figures 1 and 2.