MITOSIS

Objective:

Upon completion of this lab, the students should be able to:

1. Understand the cell cycle process and different stages of mitosis.
2. Know the importance of feulgen technique in cytological research.
3. Prepare his/her own root tip squashes of broad bean to observe its mitosis.
4. Visualize different phases of mitosis on broad beans (*Vicia faba*) root tip.
5. Describe the events during each phase of mitosis (number and structure).
6. Put the stages of mitosis in the proper order.
7. Calculate the duration of each mitotic stage.
8. Estimate the Mitotic indices in different samples.

Introduction

Eukaryotes are diploid i.e. have two set of chromosomes (2n); one set inherited from each parent. Their cells contain a nucleus and other structures (organelles) enclosed within membranes. Their nuclei are membrane-enclosed organelles with most of the cell's genetic material, organized as multiple long linear DNA molecules in complex with a large variety of proteins, such as histones, to form chromosomes. The genes within these chromosomes are the cell's nuclear genome. The function of the nucleus is to maintain the integrity of these genes and to control the activities of the cell by regulating gene expression — the nucleus is, therefore, the control center of the cell.

Mitosis is the process of nuclear division in a living cell by which the carriers of hereditary information, or the chromosomes, are exactly replicated and the two copies distributed to identical daughter nuclei (2n). Mitosis is almost always accompanied by cell division (cytokinesis), and

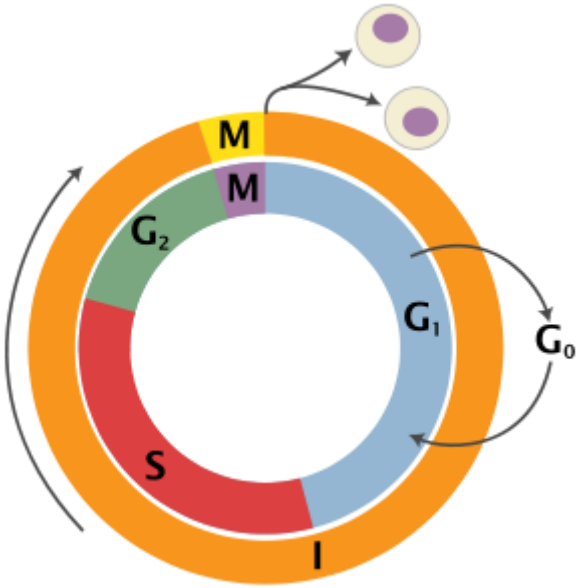
the latter is sometimes considered a part of the mitotic process. The pattern of mitosis is fundamentally the same in all cells. However, while animal cells apparently divide by pinching into two separate cells, plant cells develop a cell plate, which becomes a cellulose cell wall between the two daughter cells. The importance of mitosis is the maintenance of the chromosomal set; each cell formed receives chromosomes that are alike in composition and equal in number to the chromosomes of the parent cell (genetically identical).

Eukaryotic organisms carry out mitosis throughout their entire life to grow, develop, and asexually reproduce (some cases), as well as to renew the old and damaged cells.

Note: Cell division in eukaryotes is different from that in organisms without a nucleus (Prokaryote).

Cell cycle

The cell cycle, or cell-division cycle, is the series of events that take place in a cell leading to its division and duplication (replication). In cells without a nucleus (prokaryotic), the cell cycle occurs via a process termed binary fission. In cells with a nucleus (eukaryotes), the cell cycle can be divided in three periods: interphase—during which the cell grows, accumulating nutrients needed for mitosis and duplicating its DNA—and the mitotic (M) phase, during which the cell splits itself into two distinct cells, often called "daughter cells" and the final phase, cytokinesis, where the new cell is completely divided.



Schematic of the cell cycle. outer ring: I = Interphase, M =Mitosis; inner ring: M = Mitosis, G_1 = Gap 1, G_2 = Gap 2, S =Synthesis; not in ring: G_0 = Gap 0/Resting

Interphase

The Interphase consists of three distinct phases: G_1 phase, S phase (synthesis) and G_2 phase. Activation of each phase is dependent on the proper progression and completion of the previous one. Cells that have temporarily or reversibly stopped dividing are said to have entered a state of quiescence called G_0 phase. Cells increase in size in Gap 1 (G1). The G_1 checkpoint control mechanism ensures that everything is ready for DNA synthesis. DNA replication occurs during the S phase. During the gap between DNA synthesis and mitosis (G2), the cell will continue to grow. The G_2 checkpoint control mechanism ensures that everything is ready to enter the M (mitosis) phase and divide.

Mitosis

Cell growth stops at this stage and cellular energy is focused on the orderly division into two daughter cells. A checkpoint in the middle of mitosis (*Metaphase Checkpoint*) ensures that the cell is ready to complete cell division.

The relatively brief *M phase* consists of nuclear division (karyokinesis).It is relatively short period of cell cycle. M phase is complex and highly regulated. The sequence of events is divided into phases, corresponding to the completion of one set of activities and the start of the next. These phases are sequentially known as:

- prophase,
- metaphase,
- anaphase,
- telophase

Mitosis is the process by which a eukaryotic cell separates the chromosomes in its cell nucleus into two identical sets in two nuclei. During the process of mitosis the pairs of chromosomes condense and attach to fibers that pull the sister chromatids to opposite sides of the cell. It is generally followed immediately by cytokinesis, which divides the nuclei, cytoplasm, organelles and cell membrane into two cells containing roughly equal shares of these cellular components. Mitosis and cytokinesis together define the **mitotic (M) phase** of the cell cycle - the division of the mother cell into two daughter cells, genetically identical to each other and to their parent cell.

Prophase

The DNA molecules progressively **shorten** and **condense** by coiling, to appear as chromosomes. The nuclear membrane and nucleolus start to disintegrate (early prophase) then disappear (late prophase). The spindle apparatus has migrated to opposite poles of the cell.

Metaphase

The spindle apparatus are completely settled at opposite poles of the cell. The spindle fibers attach themselves to the kinetochore (present at both sides of centromeres of the chromosomes) and align the chromosomes at the equatorial plate.

Anaphase

The spindle fibers shorten, the centromere splits, and separated sister chromatids are pulled along behind the centromeres.

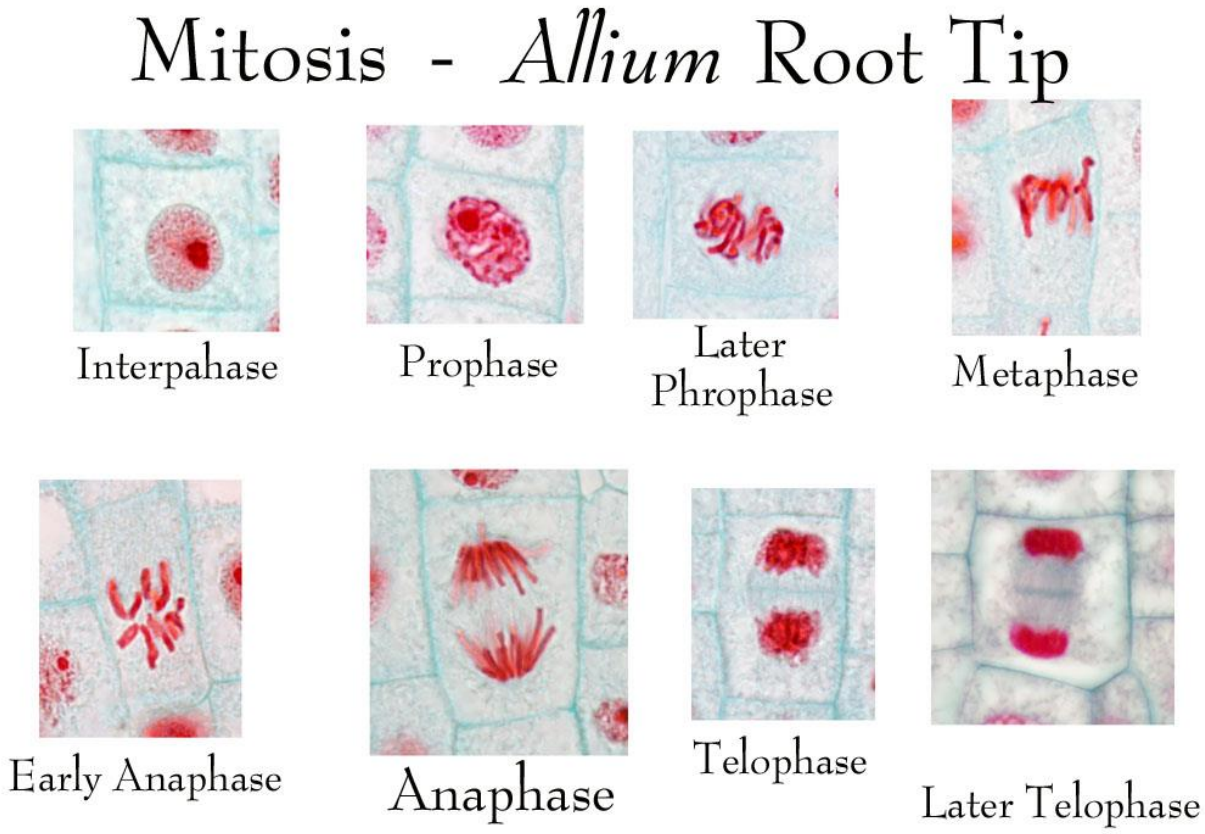
Telophase

The chromosomes reach the poles of their respective spindles. Nuclear envelope start reformation (early) and completely reformed (late).

The chromosomes uncoil. The spindle fibres disintegrate.

Cytokinesis

Cytokinesis is not part of mitosis but is an event that directly follows mitosis in which cytoplasm is divided into two daughter cells. A furrow forms and the cell is pinched in two. Each daughter cell contains the same number and same quality of chromosomes. The cell then enters interphase - the interval between mitotic divisions.



LAB WORK

Feulgen Squash Technique for Mitosis

Material

1. Previously prepared broad bean (*Vicia faba*) root tips.
2. Light microscope with 10x and 40x objectives.
3. Water bath
4. Lab coat
5. Slides and coverslips
6. Dry, clean test tubes in rack.
7. Paint brush
8. Pencil eraser
9. Razer blade
10. Paper towels
11. 1N HCl
12. 45% acetic acid
13. Feulgen stain

Chemicals preparation:

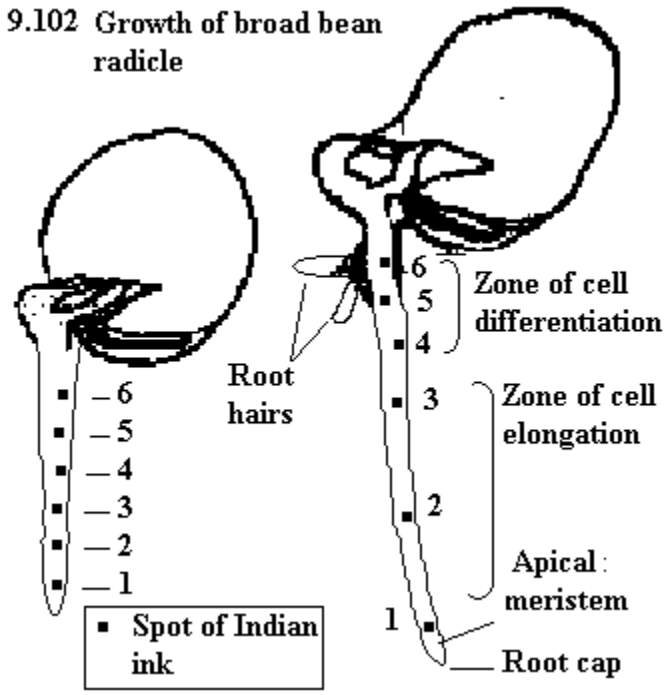
1. 1N Hydrochloric acid (HCl)
2. 45% acetic acid
3. Feulgen stain: one gram basic fuchsin was dissolved in 200 ml boiled distilled water, shake well and cool to 50-55°C. Add 30 ml 1N HCl and 3 g potassium metabisulphide ($K_2S_2O_5$) to the stain. Allow the stain to bleach for 24 hr in a dark stoppered bottle. Shake well, filter and stored the bottles in dark.

Procedure

1. Dry broad beans (uniform in size and shape) were soaked in tap water for 24 hr. soaking water must be changed every 12 hr to avoid anoxia of cells on divisions.

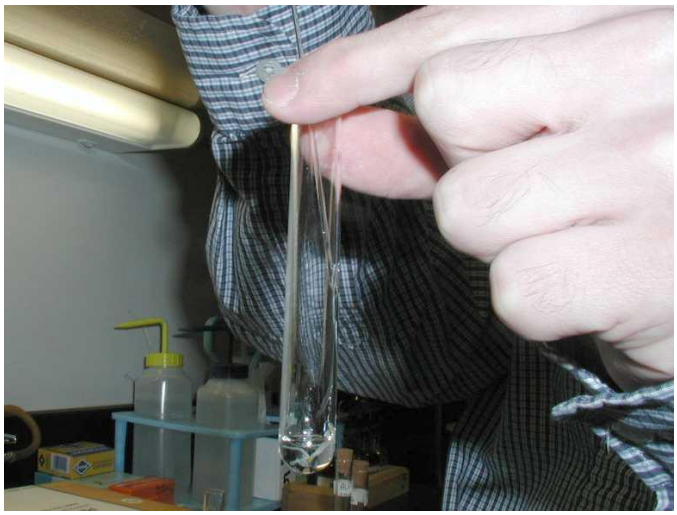
2. Seeds were sown vertically in irrigated loose sand or sawdust for 3 days. **Why loose sand or sawdust was used?**

3. Healthy straight roots (about 2 cm) were collected and washed with distilled water to remove excess sand or sawdust. **Why we take the root tip?**



4. The root tips (0.5-1 cm) were cut off and transfer immediately the root tips in Fixative solution (acetic acid:alcohol =1:3) for 24 hr, then store them in 70% ethanol in refrigerator. This process will stop the cell division or growth.

5. Wash the needed roots in test tubes 5 times for 5 min.
6. Hydrolyze the root tips in 1N HCl (test tubes in water bath) at constant 60°C for 7-9 min in separate tubes. **Why the tissues were hydrolyzed?**



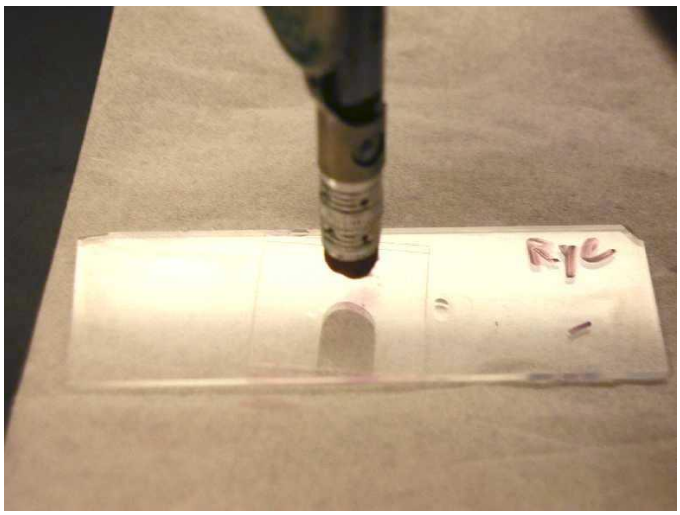
7. Pour off the fixative and wash the hydrolyzed roots in distilled water to get rid of the acid.
8. Separate the tips into 3 different labeled tubes.
9. Using the paint brush to transfer the tissue to tubes filled with 2ml feulgen reagent.
10. Stain for ½ hour in the lab with leucobasic fuchsin in dry clean tubes.

Note: This dye will stain hands and closes.

11. Remove the root tip from the stain and transfer it to a clean slide.

12. Cut the dark purple color of the meristematic region with sharp razor blade.

13. Squash the meristematic region of the root tip with the pencil eraser and add 1-2 drops of 45% acetic acid onto the slide. **Why using acetic acid on squashing?**



14. Place a coverslips over the tissue.

Note: Try to avoid air bubbles under the coverslip to prevent drying the tissue.

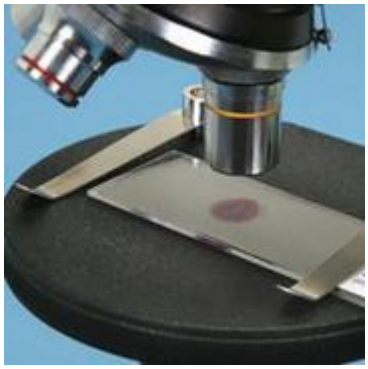
15. Press down firmly only onto the coverslip with pencil eraser to spread the cells in a very thin layer to see the divisions. The tapping should be gentle.

    Note: The coverslips should not slip or break.

16. Use the paper towels or blotting paper to remove excess acetic acid over the slide and cover slip.

17. Examine under low power (10x objective) to found areas with cells undergoing mitosis.

18. Examine under high power (40 x objective) and find all the stages.

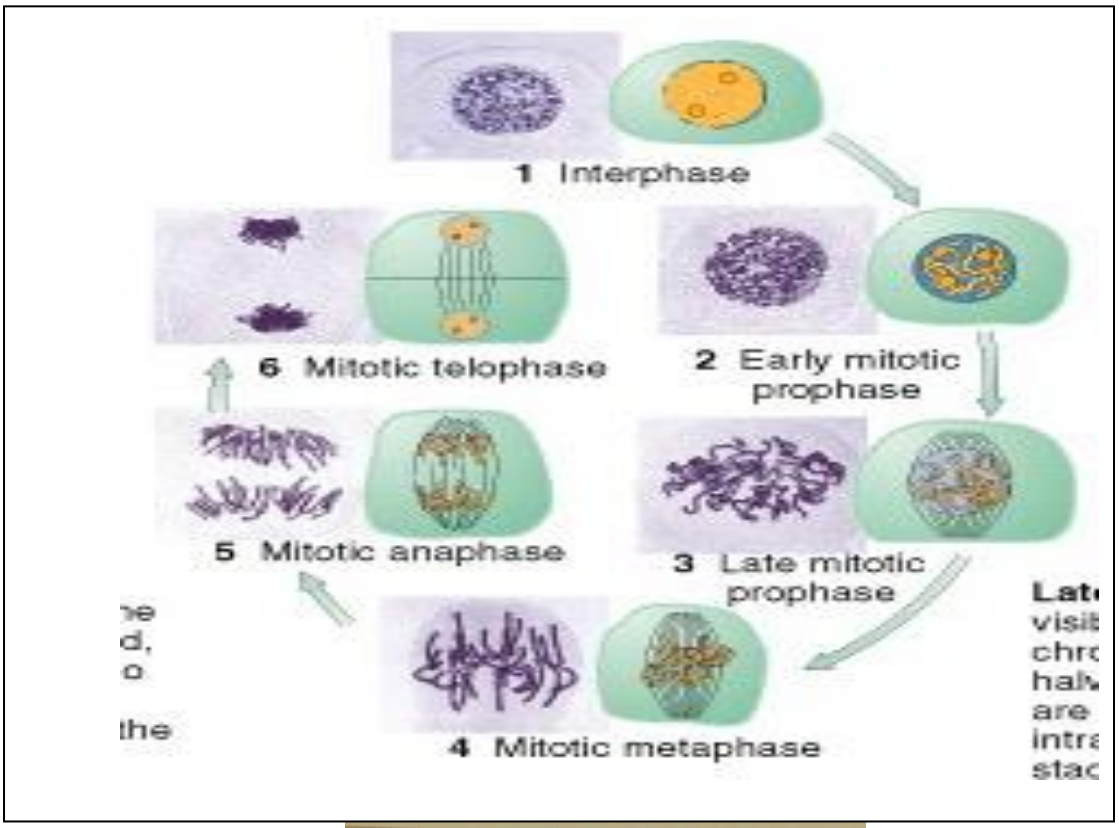


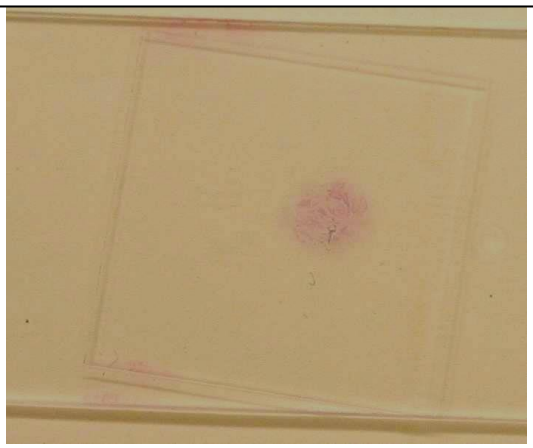
19. After you have set up your own slides, your instructor will adjust your best slides on the main lab bench pointing on the stages of mitosis.

Observation:

1. The material was spread into one layer of flattened rectangular cells.

   Note: If not you will not be able to see divisions. You will see only overlapped cells.





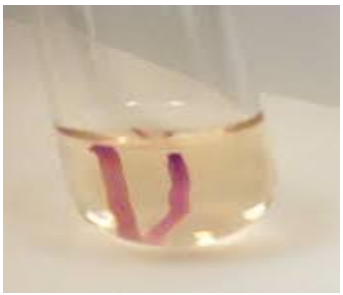
2. All the Cell cycle stages were present: Interphase, mitosis and cytokinesis.

3. All the cells appear rectangular.

   Note: If not (the cell in spindle form) your coverslip must have moved.

4. The cell must have purple color.

   Note: Optimum hydrolysis produces adequate aldehydic groups from the deoxypentose sugar of DNA to react with the staining reagent giving rise to the maximum results of the dye-PURPLE. But if faint color, it may be the effect of either less (few liberated aldehydic groups) or excess hydrolysis (destructive effect on the structure of nucleic acid).



WORK SHEET

1. Examine your prepared slide with stained root apical meristem. Which hydrolysis incubation period showed the best results?

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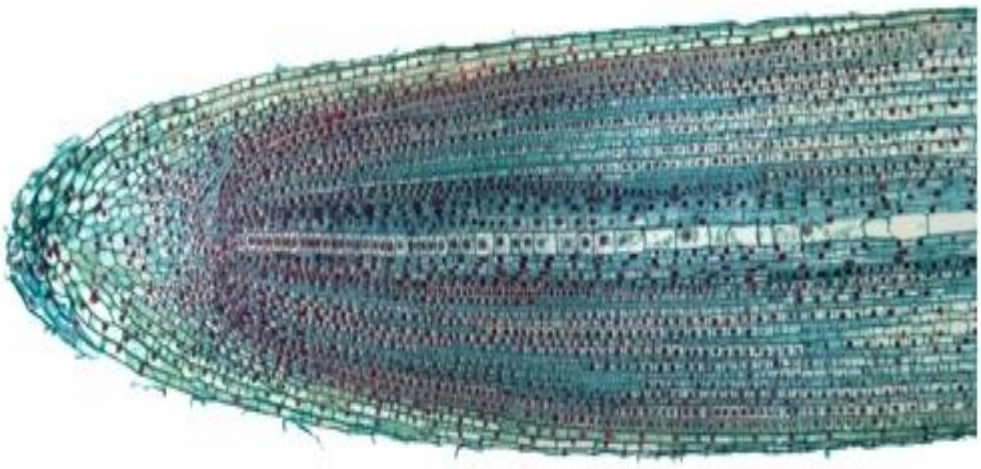
2. Examine the provided photo of the L.S. in root apical meristem. Are most of the cells dividing or is the majority in interphase?



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3. Examine your own slide under microscope. Scan your slide to find all the mitotic stages and draw them below. Indicate the chromosome number and structure of each stage.

Interphase	**Mitosis**	
	Prophase	**Metaphase**

	Anaphase	**Telophase**

4. Record the total number of cells that you can see in the field of your microscope. Calculate the percentage of each stage and its duration in the cell cycle. Where the cycle duration=1440 min (divide every 24 hr).

Duration of a stage (min) = cells in the stage for 100 cells x cycle duration (min)

Or

The duration of Stage in the cell cycle (minutes) = (number of cell in stage / total number of cells) x 1440

Stage of cell cycle	**# cells in the stage**	**Duration of the stage (min)**

Interphase		
Prophase		
Metaphase		
Anaphase		
Telophase		
Total	100 cells	

5. Quantify the cell division of the broad bean in your slide by using the mitotic index:

   Mitotic index = number of cell in mitosis/ total number of cells

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Note: For a group of cells that rarely complete the cell cycle, we expect a high proportion of cells to be in the resting stage of the cell cycle (G1). However, in a rapidly dividing cell population, we expect a high proportion of cells to be in the stage of mitosis.