GENETICS

Lab

(2018- 2019)

Please read and make sure you understand the following instructions and knowledge before you go on.

Revised the Lecture and Lab:

- The Cell cycle
- Mitosis phases

FEULGEN SQUASH TECHNIQUE FOR MITOSIS

Objective:

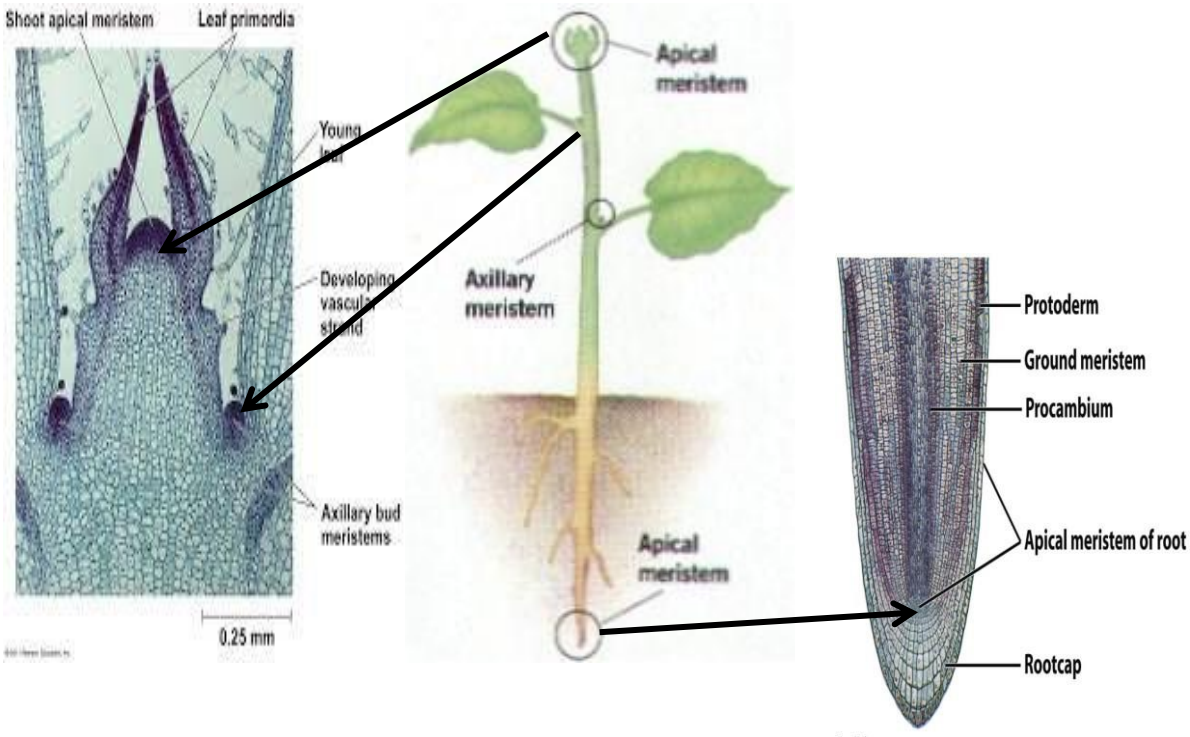
Upon completion of this lab, the students should be able to:

1. Know the importance of Feulgen technique in cytological research.
2. Prepare his/her own root tip squashing technique to observe mitotic divisions in meristematic cells of onion roots' tips.
3. Evaluate the quality of the stain in relation to hydrolysis.
4. Visualize different phases of mitosis on root tips of both broad beans (*Vicia faba*) and onion (*Allium cepa*).
5. Identify the various stages of mitosis in the root cells.
6. Sketch each mitotic stage and summarize the main events of each stage.
7. Describe the events during each phase of mitosis (number and structure).
8. Define and Value the mitotic index and the duration of each stage in the cell cycle.

Introduction

Feulgen stain is undoubtedly the most widely used staining technique discovered by Robert Feulgen. It is a semi-quantitative technique used in cyto-histology for investigating chromosomes and its DNA (DNA-specific reaction) in dividing cells depending on acid hydrolysis of DNA and be visualized with optical microscopy.

In plants, mitotic cell division mainly takes place in special regions called **meristems**. They are either present in Shoot apex or axillary buds or root tips of the plants for development and growth.



MATERIALS AND METHOD

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. Razor blade
10. Paper towels

Chemicals preparation

1. **1N Hydrochloric acid (HCl):** If your stock concentration came in 12M (smocking), then using the following equation:

$$\mathbf{N\ V = N1\ V1}$$

$$\text{So, } V = (N1\ V1) / N$$

Where,

V = volume of your stock concentration, N = concentration of your stock

V1 = volume of your final preparation, N1 = concentration of your final

$$V = (1\ N \times 100\ ml) / (12N) = 8.3ml$$

2. **45% acetic acid:** 45 ml of glacial acetic acid rises up to 100 ml with distilled water.

3. **Feulgen stain (Schiff's reagent)**: 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 to the stain then 3 g potassium metabisulphide ($K_2S_2O_5$). Allow the stain to bleach for 24 hr in a dark stoppered bottle. Shake well, filter with charcoal and stored the bottles in dark.

Procedure

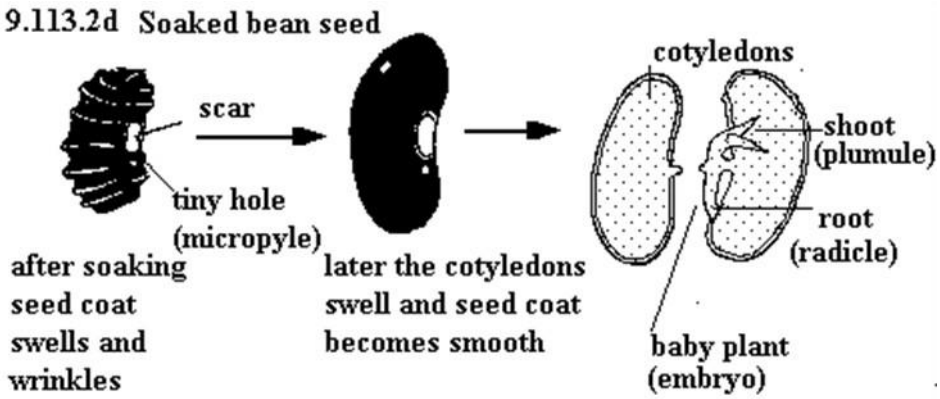
For Onion:

1. Choose the onion with no injury at the base.

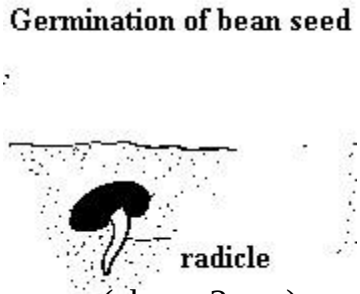
2. Submerge the base of an onion in water at constant temperature.

For Broad bean:

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. **Why?** 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?** So, the root will not rupture by harvesting.



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? To see mitotic divisions as it consist of meristematic dividing cells.

General steps:

1. 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. **Why we do this process?** The combination of these two chemicals leaves the chromosomes in a highly precipitated form suitable for staining and microscopic study by preserving their structural and/or chemical components i.e. stop the cell division and growth.

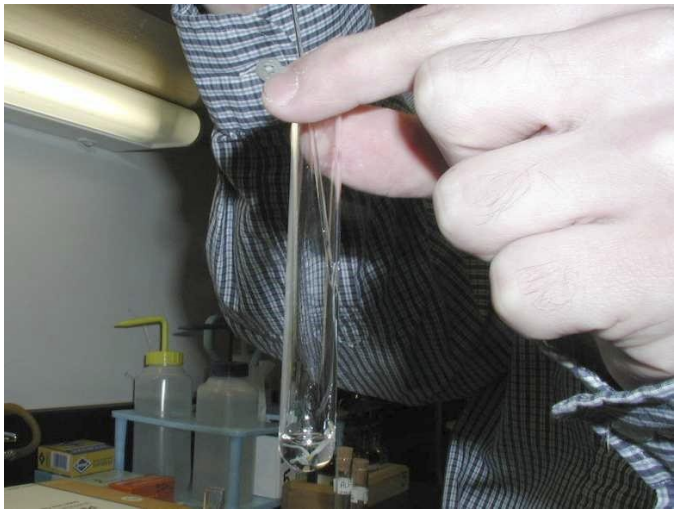
2. Wash the needed roots in test tubes 5 times for 5 min. **Why?** to remove excess fixative.

3. Separate the tips into 3 different labeled tubes.

4. Hydrolyze the root tips in 1N HCl (test tubes in water bath) at constant 60°C for 7, 8 and 9 min for beans and 4,5and 6 min for onion in separate tubes. **Why the tissues were hydrolyzed?**

1.To separate the cells from each other to see the division clearly.

2. To break the purine-base glycoside linkage yielding free aldehyde group from deoxypentose sugar along DNA.



5. Pour off the fixative and wash the hydrolyzed roots in distilled water 3 times to get rid of the acid (check that the odour of acid is removed).

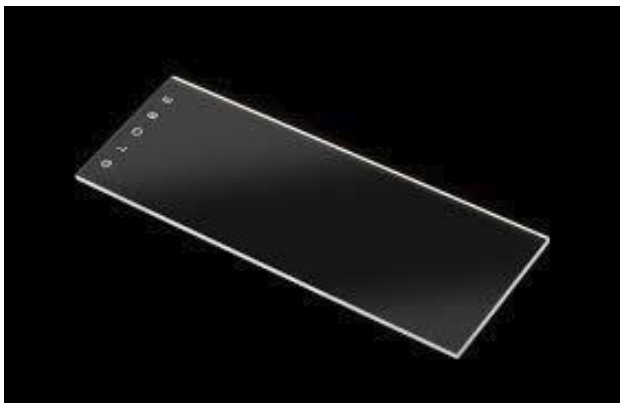
6. Using the paint brush to transfer the tissue to tubes filled with 2ml feulgen reagent (Schiff reagent). **Be careful that the roots are now very fragile.**

7. Stain for ½ hour in the lab with leucobasic fuchsin in dry clean tubes. **What happened?**

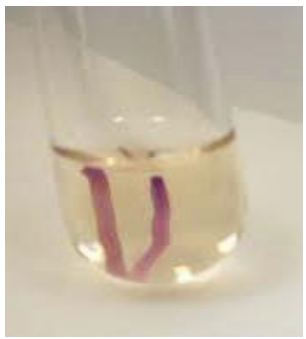
The Schiff reagent is prepared by adding 1N HCl (acid) and potassium meta-bisulphite (salt) to an aqueous solution of basic fuchsin. This addition causes the liberation of free SO_3 which reduces the purple color of the basic fuchsin into a colorless solution (leucobasic fuchsin). The reagent reacts with the free aldehydic groups (CHO) from the deoxypentose sugar of DNA producing a DNA specific insoluble magenta colour compound.

Note: Take care this dye will stain hands and closes.

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



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



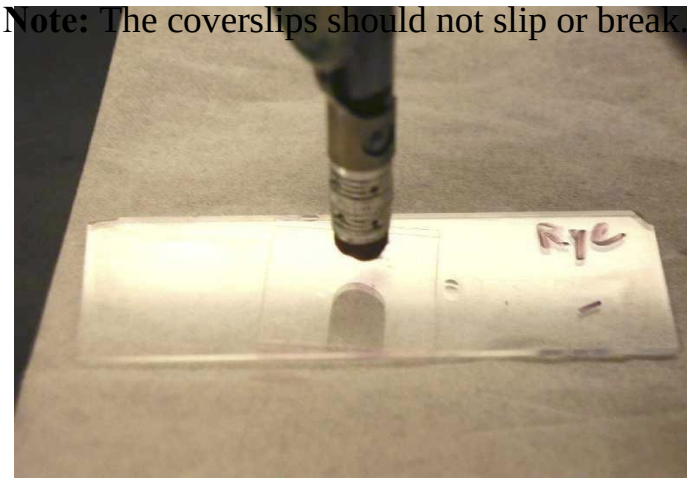
10. Squash the meristematic region of the root tip with the pencil eraser and add 1-2 drops of 45% acetic acid onto the slide. **Why using acetic acid on squashing?** To easily spread the cells in a very thin layer to see the divisions clearly.

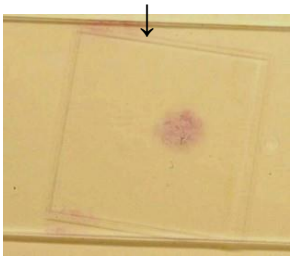
11. Place a coverslips over the tissue.

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

12. Press down carefully onto the coverslip with pencil eraser to spread the cells in a very thin layer to see the divisions. The tapping should be gentle as the hard way will split the cover.

Note: The coverslips should not slip or break.

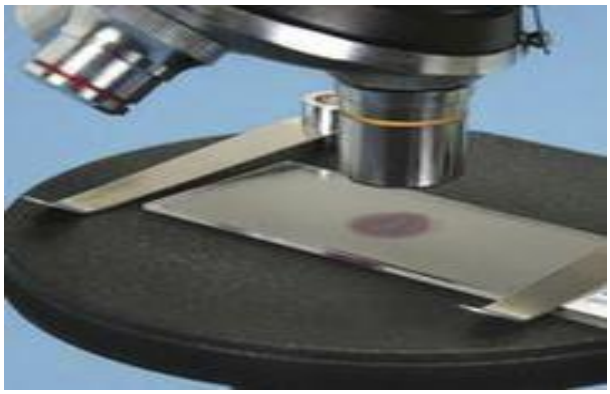




13. Use the paper towels or blotting paper to remove excess acetic acid over the slide and cover slip.

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

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

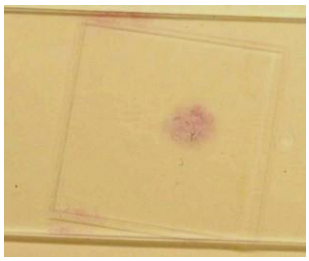


16. After you have set up your own slides, your instructor will adjust your best slides on the main lab bench pointing on the stages of mitosis.

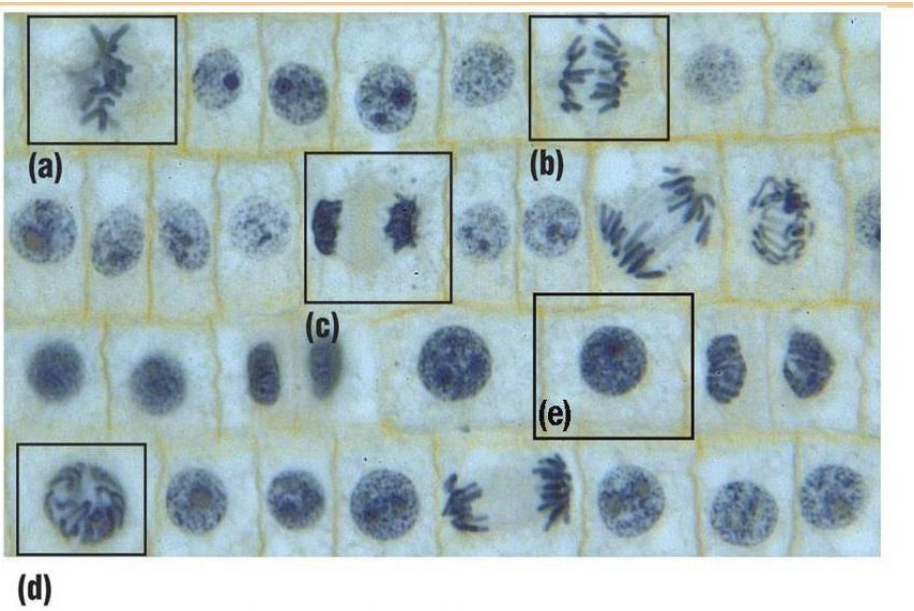
Observation:

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.



a. Metaphase, b. Anaphase, c. Telophase, d. Prophase and e. Interphase

3. All the cells appear rectangular.

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

4. The best cell stain must have **purple color**.

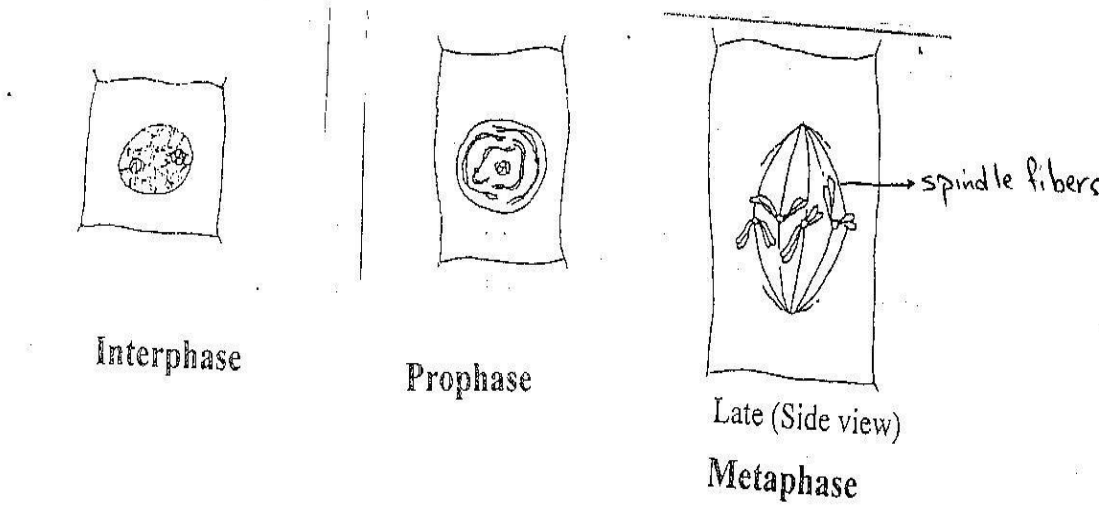
Why?

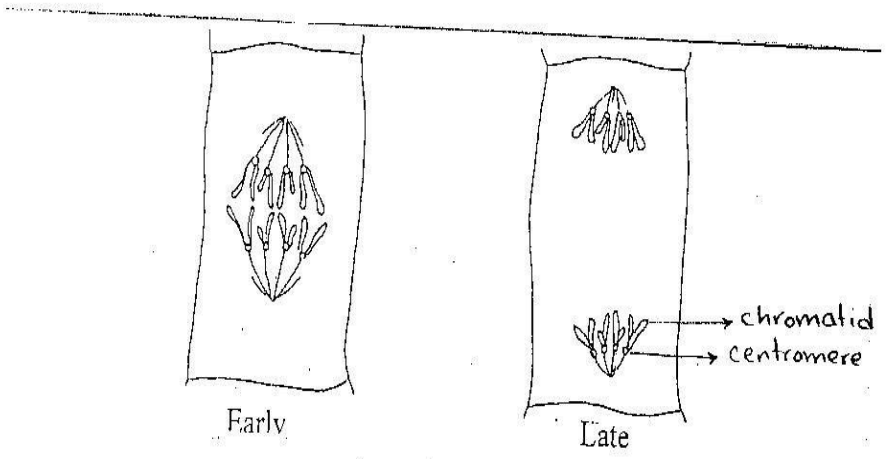
Optimum hydrolysis liberates adequate purine and aldehydic groups from the deoxypentose sugar of DNA to react with the staining reagent giving rise to the maximum results of the dye (**purple color)**.

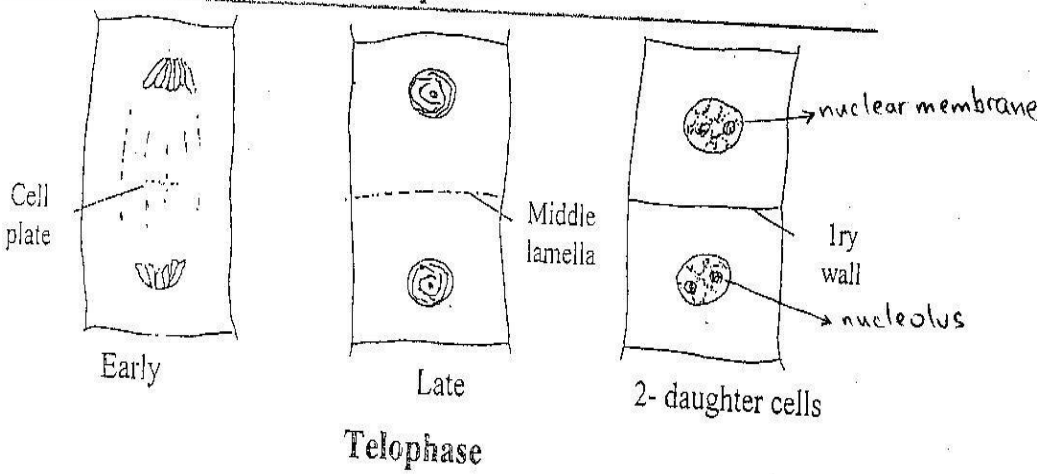
But if **faint color**, it may be the effect of either:

- less hydrolysis due to the few liberation of purine and aldehydic groups

- or excess hydrolysis due to destructive effect on the structure of nucleic acid by removing both histones (protein associated with DNA) and apurinic acids (deoxyribose without the base) .







Stages of mitosis