Core practical 3: Observe mitosis in root tips

Objectives

- Know how to prepare a temporary slide of a root tip to observe mitosis
- Recognise the stages of mitosis in dividing cells
- Identify hazards, associated risks and control measures for the procedure

Safety

- Eye protection must be worn.
- Take care with glassware and scissors.
- Acetic orcein stain is corrosive, causes burns, has an irritating vapour and will stain. Wear eye protection and avoid contact with skin. If contact does occur, wash the area thoroughly with water for 10 minutes. Mop up spillages immediately.

Specification links

- Practical techniques 3, 4, 5, 8, 10
- CPAC 1a, 2a, 2b, 3a–3c, 4a, 4b

Procedure

To see mitosis in action you need to look at living cells. Garlic bulbs grow roots that have actively dividing cells in their tips, in a region called the meristem. Each cell has only eight chromosomes so it is relatively easy to see the chromosomes once they have condensed. In order to see the chromosomes inside the cells, the cells must be separated and spread out into a layer that is ideally just one cell thick. Plant cells are glued together by a middle lamella of pectins. Hydrochloric acid will break down the pectins that hold the cell together. Acetic orcein will stain the chromosomes dark red and fix the cells, stopping mitosis.

You should examine your preparation carefully for cells undergoing different stages of mitosis. Identify the different stages by comparing your preparation with labelled pictures or photographs of cells during mitosis. Bear in mind that mitosis is a dynamic process so cells may have been fixed in transition from one stage to the next – you will have to interpret what you see.

Make sure that all safety precautions given on this sheet are followed carefully. You should also have completed your own risk assessment prior to this practical.

1. This first step may have been done for you. Fill a small bottle with 1 mol dm^{-3} hydrochloric acid, and place it in a thermostatically controlled water bath set at 55 °C. Leave the bottle for 15 minutes to let the acid warm to the temperature of the water bath.
2. Place a garlic clove in the top of the bottle so that the roots are submerged in the hydrochloric acid at 55 °C. Leave the roots in the acid for 5 minutes.
3. After 5 minutes, take the clove out and rinse the roots thoroughly in tap water. Use a pair of sharp scissors to cut off several root tips of 5–10 mm in length. Let them fall into a small vial of acetic orcein standing on a white tile. Use the scissors to make sure that the root tips are immersed in the stain. Place a lid or laboratory stretch film onto the vial. Lids should have a pin-prick hole, or should be slightly loose if they are screw caps, to prevent the ejection of liquid when heating.
4. Place the vial containing root tips in acetic orcein in the 55 °C water bath for 5 minutes to intensify the staining.
5. After 5 minutes, use forceps to take the tips out of the vial, and place them on a microscope slide. Add a drop of water to the root tip on the slide. Tease the root tip apart with needles (maceration), to spread out the cells a little. Cover with a coverslip. Replace the lid on the vial of stain and return it to the teacher as instructed.
6. Wrap the slide in several layers of paper towel and press gently on the paper to squash the tissues. Take care not to twist the slide as you press down or the coverslip will break.

7. Examine under the microscope on low power to identify the area of dividing cells or meristem (see **fig A** in the Student sheet). Position the cells in the centre of the field of view. Meristem cells are small and square, have no obvious vacuoles and are usually found in rows.
8. Move to high power (×400). Identify as many stages of the cell cycle as you can in your field of view.
9. Count the number of cells in each of the stages of mitosis, plus interphase, in the field of view. Record your results in a table.
10. Draw and annotate one cell from each of the stages you have identified. Your drawings will be simple outlines of the cells and the groups of chromosomes in them; few other structures will be visible. Aim to show the relative sizes and positions of the chromosomes and the cell accurately. Annotate your drawings to describe what is happening.

Notes on procedure

- This practical should be used as an opportunity to develop risk assessment skills. Students will need guidance on how to do this. They should identify hazards and consider these, along with the likelihood of problems, to assess risk. They should suggest suitable control measures to reduce risk. Often a table is the best way to present this. Ideally, students should be provided with a brief outline of the practical and information about reagents prior to the activity and asked to produce their own risk assessment. The safety guidance on the official practical sheet should then be followed after comparison.
- Even though it is more hazardous, acetic orcein stain is suggested here in preference to toluidine blue O because the stain gives better definition of chromosome structure. Using a vial for staining reduces the risk of spillage.
- A common mistake is that students mix up the tip and the non-dividing end of the root.
- Heating with a mixture of acetic orcein stain and HCl is a potential hazard and careful checks must be made as the students attempt this. Pressing on the paper and slide must be done with even pressure. Any quick or twisting movements will crack the coverslip.
- Ensure students view the preparation under the microscope on low power first to identify the area of dividing cells and to focus. Higher power will be required to examine the dividing cells for the stages of mitosis.
- It is advisable to have some permanent slides available for those students who do not manage to produce a good-quality temporary slide.
- There is scope for further investigations within this practical. Students could investigate the mitotic index of roots of different ages, for example.
- For further information, see CLEAPSS Teaching and learning guide TL015, A safe and effective small-scale method for preparing and staining root tips to show chromosomes (updated 2015).

Answers to questions

1. The root tip is heated with acid to break up the tissues into individual cells. The cellulose walls of plant cells are held together by pectins such as calcium pectate. Treatment with hydrochloric acid breaks this down.
2. Pressing the preparation will separate the cells in the meristem tissue into individual cells in a single layer. This makes it easier to see the chromosomes and to identify the stages of division.
3. The cell counts show the relative duration of each stage in the cell cycle. The longer a phase, the more cells are likely to be going through that phase at any point in time.
4. Mitosis produces identical daughter cells for growth, replacement and repair.

Sample data

Phase	Number of cells	Time spent by cells in each phase (%)
interphase	112	82
prophase	14	10
metaphase	4	3
anaphase	3	2
telophase	4	3

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All the maths you need

- Use ratios, fractions and percentages.
- Solve algebraic equations.

Equipment

- garlic clove with growing root tip
- glass slide and coverslip
- scissors
- water bath at 55 °C
- small bottle with lid or laboratory stretch film
- hydrochloric acid 1 mol dm^{-3}
- acetic orcein stain in a small bottle or vial
- two dissecting needles
- paper towels
- microscope
- white tile
- fine forceps
- stop clock
- safety information sheet

Diagram

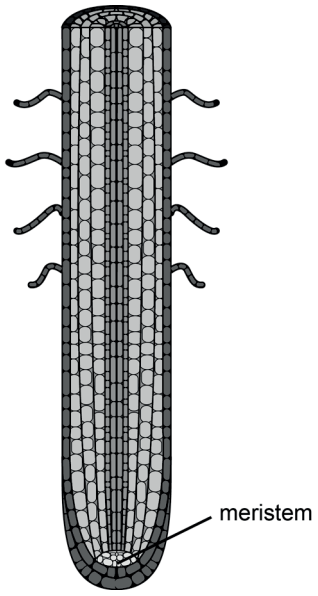

fig A Section of a root tip showing the meristem with dividing cells

Procedure

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7. Examine under the microscope on low power to identify the area of dividing cells or meristem (see **fig A**). Position the cells in the centre of the field of view. Meristem cells are small and square, have no obvious vacuoles and are usually found in rows.
8. Move to high power (×400). Identify as many stages of the cell cycle as you can in your field of view.
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Analysis of results

1. Calculate the percentage of the cells in each stage by dividing the number of cells in one phase by the total number of cells and multiplying by 100. Add the percentages to your table. You could use a spreadsheet to calculate and record these values. Given that your preparation freezes the process of mitosis at one point in time, what do these values suggest to you about the length of time a cell spends in each stage of mitosis?
2. If a group of cells is dividing rapidly, a high proportion of the cells will be undergoing mitosis. A group of cells that is not dividing will have all cells in interphase of the cell cycle (where chromosomes will not be clearly visible). The amount of cell division occurring in a tissue can be quantified using the mitotic index. The mitotic index is used for studying tumour growth in cancer patients. Using the formula below, calculate the mitotic index for your root tip.

$$\text{mitotic index} = \frac{\text{number of cells containing visible chromosomes}}{\text{total number of cells in the field of view}}$$

Learning tips

- Cell counts of each stage of mitosis in the field of view should give evidence of the duration of each of the stages. This will be a relative value – the more cells you can see in one stage, the longer the duration of that stage in the cell cycle.

Questions

1. Explain why the root tip is heated with acid.
2. What effect will maceration and pressing the slide preparation have on the dividing cells?
3. What information do the cell counts give you about each stage of mitosis?
4. What is the role of mitosis in the life of an organism?

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Safety
● Eye protection must be worn. ● Hazard warnings on use of sharps such as glassware and needles must be given. ● Acetic orcein is corrosive, causes burns, has an irritating vapour and will stain.

Equipment per student/group	Notes on equipment
garlic clove with growing root tip	One per group. Younger, newly emerged roots of less than 1 cm produce the best results. Garlic cloves will produce roots when suspended individually with the blunt end in water, e.g. at the top of a test tube. It takes about 2–3 days for suitable root growth at 25 °C.
glass slide and coverslip	One per group
scissors	One pair per group
water bath at 55 °C	With supports for the small bottles of acid and vials of stain. Placing the vials in a shallow layer of water in a large beaker supported in the water bath means that the vial stays upright.
small bottle, e.g. universal bottle with lid or laboratory stretch film	These bottles could be filled with the 1 mol dm^{-3} hydrochloric acid and placed in the water bath in advance of the lesson. The bottles should be of such a size that a garlic clove can be placed in the top with the roots reaching into the acid.
hydrochloric acid 1 mol dm^{-3}	HCl is a low hazard at this strength. At 2 mol dm^{-3} the acid is an irritant. It is corrosive at a higher molarity than 6.5 mol dm^{-3}.
acetic orcein stain in a small bottle or vial, e.g. universal bottle or McCartney bottle	One vial of stain per student or group. Stock solution is corrosive. Wear eye protection and chemical-resistant gloves and use a fume cupboard when diluting. The diluted solution of 10 ml stock to 12 ml water is also corrosive and does not store well. The hazards of using the acetic orcein stain are very much reduced by dispensing a small volume of the stain into a bottle for students. Bottles require pop-on lids, screw-on lids or laboratory stretch film to contain the stain. Lids will need a pin-prick hole or should be left slightly unscrewed when heating to prevent ejection. Place a small volume of the stain (approximately 2 mm depth) in each bottle and label with a hazard warning.
dissecting needles	Two per group

paper towels	Several per group
microscope	One per group. Magnification of ×400 will be needed.
white tile	One per group
fine forceps	One pair per group
stop clocks	One per group
permanent slides of mitosis	These should be made available for students who do not manage to produce a good-quality temporary slide.
safety information for students to examine	For example, provide information about acetic orcein and hydrochloric acid.

Notes