H2 ANDERSON SERANGOON JUNIOR COLLEGE
HIGHER 2

2023 JC2 PRELIMINARY EXAMINATION

CANDIDATE NAME	
CLASS	INDEX NUMBER

BIOLOGY 9744/04

Paper 4 Practical

30 August 2023
Wednesday
2 hours 30 minutes

Candidates answer on the Question Paper.
Additional Materials: As listed in the Confidential Instructions.

READ THESE INSTRUCTIONS FIRST

Write your name, class and index number on all the work you hand in.
Give details of the practical shift and laboratory, where appropriate, in the boxes provided.
Write in dark blue or black pen.
You may use an HB pencil for any diagrams or graphs.
Do not use staples, paper clips, glue or correction fluid.

Answer **all** questions in the spaces provided on the Question Paper.

The use of an approved scientific calculator is expected, where appropriate.
You may lose marks if you do not show your working or if you do not use appropriate units.

At the end of the examination, fasten all your work securely together.

The number of marks is given in brackets [ ] at the end of each question or part question.

Shift
Laboratory

For Examiner's Use	
1	
2	
3	
Total	/55

This document consists of **15** printed pages and **1** blank page.

Answer **all** questions.

1 Plant cells contain enzymes that catalyse metabolic reactions. Some of these enzymes catalyse the release of oxygen from hydrogen peroxide.

A plant extract solution can be produced which will contain these enzymes.

When hydrogen peroxide and a plant extract solution are mixed, oxygen is released. The oxygen forms bubbles that make a foam on the surface.

You are required to investigate the effect of pH on the progress of the enzyme-catalysed reactions by:

- changing the pH using buffers
- measuring the height of the foam produced by the release of oxygen.

To follow the progress of this reaction, you will need to measure the height of foam at different times for a total of 3 minutes.

(a) (i) Decide how often you will take these measurements, including the final height of foam at 3 minutes.

State the times when you will measure the height of foam. [1]

Must state **at least 5** timings to measure, at **regular intervals → preferred: 30s, 60s, 90s, 120s, 150s, 180s [must]**

(ii) Fig. 1.1**A** shows how a test-tube will be set up at the start (0 minutes).

Fig. 1.1**B** shows the test-tube after 3 minutes.

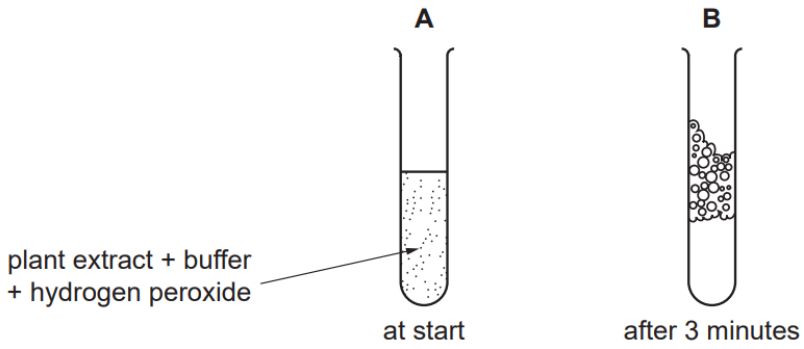


Fig. 1.1

The top of the foam may not form an even layer, so you will need to decide where to measure the layer of the foam.

Draw a double-headed arrow ↕ on Fig. 1.1**B** to show where you will measure this layer of foam. [1]

1. top and bottom of arrow to appropriate height;

You are provided with:

labelled	contents	volume / cm^3
E	plant extract solution	40
H	hydrogen peroxide solution	70
B4	buffer pH4	10
B5	buffer pH5	10
B7	buffer pH7	10
B9	buffer pH9	10
B10	buffer pH10	10
U	unknown buffer	10
D	liquid detergent	10

If any of these liquids come into contact with your skin, wash off immediately under cold water. It is recommended that you wear suitable eye protection.

Temperature affects the rate of an enzyme-catalysed reaction and may be a significant source of error.

You are required to measure the temperature of the room before the start of the investigation and when all the measurements have been recorded.

Proceed as follows:

Read step 1 to step 9.

1. Measure the temperature of the room and record this measurement in **(b)(iii)** on page 6.

2. Put 1 cm^3 of buffers **B4, B5, B7, B9, B10** into separate test-tubes.

3. Put 2 cm^3 of **E** into each test-tube. Gently shake to mix well.

4. Put 1 drop of **D** into each test-tube. Do **not** mix.

*The reaction will start as soon as **H** is added to the mixture of **E** and buffer.*

5. Put 5 cm^3 of **H** into **one** of the test-tubes.

6. Immediately start timing.

7. Using the strip of graph paper provided, measure the height of the foam as decided in **(a)(ii)**, at each of your sampling times as stated in **(a)(i)**, until you have recorded the final measurement at 3 minutes. Record your measurements in **(b)(i)**.

If the foam flows over the top of the test-tube, **stop timing**. Record the measurement of the height of the foam to the top of the test-tube **and** add an asterisk (*) to show that the foam has flowed over.

8. Repeat step 5 to step 7 with each of the other buffer solutions.

9. Measure the temperature of the room now that all the measurements have been recorded. Record the temperature in **(b)(iii)**.

(b) **(i)** Record your results for the height of foam in an appropriate table below. [4]

pH	Height of foam at specific time interval / mm					
	30s	60s	90s	120s	150s	180s
4	0	0	2	3	3	3
5	2	2	3	3	4	4
7	4	6	6	6	8	8
9	4	4	5	5	5	6
10	3	4	4	4	4	4

1. table drawn and correct headings: pH and height of foam/mm ;
2. records results for 5 pH values and all time intervals stated in (a)(i);
3. height of foam recorded as whole mm ;
4. correct trend → maximum height of foam at 180s at pH 7 ;

(ii) Using your raw results for **pH 5**:

- State the highest height of foam ..... mm.
- State the first time when this height was reached ..... seconds.

Using these measurements, calculate the rate of production of oxygen, as millimetres per second (mm s^{-1}).

Show your working.

1. shows maximum height of foam for pH 5 divided by time ;
2. answer to the appropriate degree of accuracy (to 2 or 3 sf) ;

rate of oxygen production = ... mm s^{-1} [2]

(iii) State the temperature of the room before the start of the investigation.

..... °C

State the temperature of the room after all the measurements have been recorded.

..... °C

State the difference between this temperature and the temperature of the room at the
start of the investigation.

difference = ... °C

Explain whether temperature is a significant source of error in this investigation. [1]

unlikely a significant source of error because **did not differ significantly/ temperature was the same throughout** (and independent and dependent variable was not temperature) + **correct recording of temperature to 1 d.p**.

You are now required to estimate the pH of the buffer in **U** using the same procedure.

10. Put 1 cm^3 of **U** into a test-tube and repeat step 3 to step. Record your results in **(b)(iv)**.

(iv) Record the maximum height of foam using **U**.

1. states the height of foam for U in mm, to whole number;

..... mm [1]

(v) Using the result in **(b)(iv)** and the results in **(b)(i)**, estimate the pH of U.

1. estimates the correct pH using the result stated in (b)(iv), in comparison with maximum heights of foam for each buffer of known pH after 3 minutes in results table in (b)(i) (U is actually pH 5 → accept range between 4 to 7)

pH ..... [1]

(vi) This procedure investigated the effect of pH on the activity of the enzymes in a plant extract solution.

To modify this procedure for investigating the effect of substrate concentration on the activity of enzymes in the plant extract solution, the pH should be kept the same.

You will plan but not carry out a dilution of 5.0% hydrogen peroxide solution using distilled water to reduce its concentration. You are required to make up at least 10 cm^3 of each diluted solution.

Describe and show how you will prepare the range of different concentrations of hydrogen peroxide. [3]

1. Describe **use of syringes + beakers** + concentrations of hydrogen peroxide prepared by **simple dilution or serial dilution**;
2. states at least five concentrations of hydrogen peroxide;
3. show dilution table

concentration of hydrogen peroxide solution / %	volume of 5.0% hydrogen peroxide solution / cm^3	volume of distilled water/ cm^3
1.0	2.0	8.0
2.0	4.0	6.0
3.0	6.0	4.0
4.0	8.0	2.0
5.0	10.0	0.0

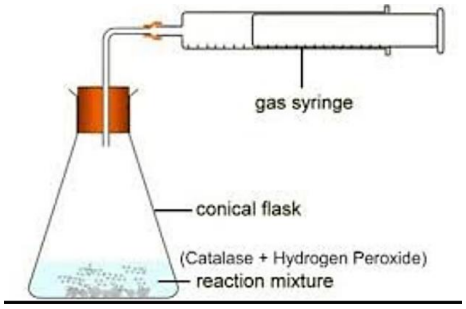
OR

	hydrogen peroxide solution				
percentage concentration of hydrogen peroxide solution	5.0	0.5	0.05	0.005	0.0005
percentage concentration hydrogen peroxide solution to be diluted		5.0	0.5	0.05	0.005
volume of the hydrogen peroxide solution to be diluted / cm^3		2.0	1.0	1.0	1.0
volume of distilled water to make the dilution/ cm^3		18.0	9.0	9.0	9.0

[1]

(vii) Describe how the dependent variable could be measured more accurately than measuring the height of foam. You *may* use a labelled diagram in the space provided.

1. describes counting the number of bubbles or collecting the volume of oxygen using displacement of water or alternative correct method;



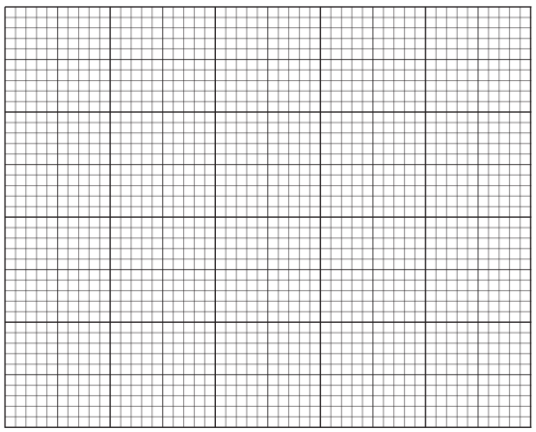
(c) A student investigated the effect of temperature on the rate of enzyme activity in a plant extract solution. All the other variables were standardised.

The results are shown in Table 1.1.

Table 1.1

temperature / ^{o}C	rate of enzyme activity / arbitrary units
5	6.5
16	39.0
27	31.0
38	9.5
49	1.0

(i) Plot a graph of the data shown in Table 1.1.



[4]

G1. Correct axes:
- x-axis – temperature / ^{o}C
- y-axis - rate of enzyme activity / arbitrary units

G2. Correct scale:
- x-axis **–** 10 ^{o}C to 10 small squares (axis interval labels to whole number)
- y-axis – 10.0 arbitrary units to 10 small squares (axis interval labels to 1 d.p)

G3. Correct plotting of all points using small crosses

G4. Points joined with a straight ruled line joining each point to the next

(ii) Use your graph to estimate the rate of enzyme activity at 32 ^{o}C. Show on your graph how you obtained your data.

…….. arbitrary units [1]

(iii) Explain the differences in the effects of changing pH and temperature on the rate of enzyme-catalysed reactions. [2]

1. R group interactions disrupted: beyond optimum temperature → H bonds, ionic bonds and hydrophobic interactions, away from optimum pH → H bonds, ionic bonds
2. Before and beyond optimum pH → enzymes denatured, before optimum temperature enzymes inactive but beyond optimum temperature, enzymes denatured.
3. Enzymes can function only in a narrow range of pH but can function in a larger range of temperature.

[Total: 22]

2 You are required to plan, but not carry out, an investigation into the effect of different wavelengths of light on the rate of photosynthesis in two different species of garden plant, Plant **A** and Plant **B**. Both plants differ in their ability to produce chlorophyll b which absorbs mainly red and blue-violet light.

Plant **A** Normal wild type (presence of chlorophyll b)
Plant **B** Chlorophyll b deficient

The rate of photosynthesis can be determined by measuring the time taken for blue DCPIP to decolourise. DCPIP is a blue dye, which acts as a hydrogen ion and electron acceptor. As DCPIP accepts hydrogen ions or electrons, it is reduced and becomes colourless.

These are the equipment and materials, which you must use in your plan.

- filtered leaf extracts from plant A and plant B
- a bench lamp
- blue, yellow and red colour filters
- 1% DCPIP solution
- capillary tubes (50 mm in length)
- stopwatch
- petri dishes
- white tile
- ruler
- aluminium foil
- glass rods

You can also select from the following apparatus in your plan:

- a variety of different sized beakers, measuring cylinders or syringes for measuring volume
- teat pipettes

(a) Given the blue, yellow and red colour filters, complete the table below to show how to produce the key wavelengths of visible light (red, orange, yellow, green, blue, violet).

You may experiment with the colour filters and lamp provided. [2]

colour	**colour filter(s) used**	**wavelength / nm**
red	red only	625
orange	red + yellow	590
yellow	yellow only	565
green	blue + yellow	520
blue	blue	435
violet	blue + red;	380

(b) Plan an investigation into the effect of different wavelengths of light on the rate of photosynthesis in Plant A and Plant B.

Your plan should:

- have a clear and helpful structure such that the method you use is able to be repeated by anyone reading it [11]

- be illustrated by relevant diagram(s), if necessary
- identify the independent and dependent variables
- describe the method with the scientific reasoning used to decide the method so that the results are as accurate and repeatable as possible
- include layout of results tables and graphs with clear headings and labels
- use the correct technical and scientific terms
- include reference to safety measures to minimise any risks associated with the proposed experiment.

Answer:

1. In the **light dependent reaction** of photosynthesis, light is absorbed by the **photosynthetic pigments** of the leaves to excite electrons from photosystems I and II.

2. Electrons released by the activated photosystems can be accepted by DCPIP which becomes reduced and decolourise/ turn colourless. Since an action spectrum is a record of the **rate of photosynthesis** occurring at **each wavelength of light,** rate of photosynthesis can be determined by the time taken for DCPIP to decolorise.

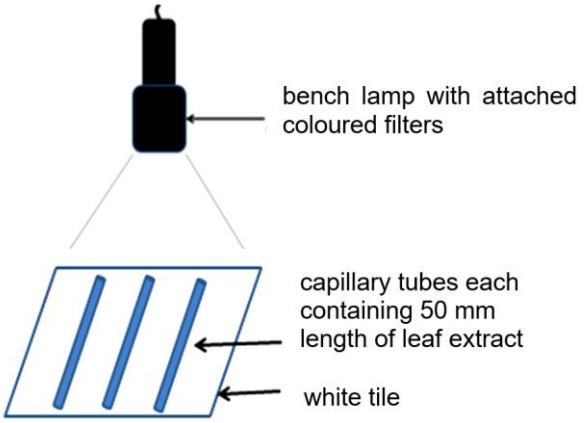
3. The higher the absorption of light, the greater the number of electrons released from the photosystems and hence the shorter the time taken to decolourise the DCPIP.

4. Species A should have a **higher rate of photosynthesis** at the red and blue-violet light with lower rate in the green light/ Species B should have an overall lower photosynthetic rate at the same wavelengths due to the lack of chlorophyll b.

5. Dependent variable: Rate of photosynthesis as measured by time taken for the decolourisation of DCPIP or 1/time taken. Unit - s^{-1}; Independent variable: wavelengths of light. unit – nm.

6. Stir the contents of the boiling tube with a glass rod before pouring a fixed volume (e.g. 5 cm^3) of the leaf extract of Species A into a petri dish.
7. Take a capillary tube and stand one end of the tube into the leaf extract. Some of the liquid will rise up into the capillary tube. Ensure that the extract fill up the entire capillary tube/ 50 mm. Place the capillary tube on a white tile and cover it with aluminium foil.
8. This serves as a colour standard to confirm whether DCPIP has been decolorized in presence of different wavelengths of light in the other tubes.
9. Using a teat pipette, add **2 drops** of 1% solution of DCPIP to the leaf extract in the petri dish and mix well with a glass rod. (Accept more than 2 drops as long as the number is fixed and specified.)
10. Repeat step 2 with a new capillary tube to draw up the leaf extract+DCPIP mixture.

11. Place this capillary tube containing the leaf extract+DCPIP onto the same white tile from step 2.
12. Completely cover the capillary tubes and petri dish with aluminum foil to shield the contents from light.
13. Prepare a second capillary tube in the same way/ following steps 4 – 7. This is the **replicate**.
14. Repeat step 4 – 7 to create a control tube, but do not remove the aluminum foil at all. This is to serve as a control to show that the effect of DCPIP decolorizing is due to the effects of light being used for photosynthesis.
15. Using a ruler, **fix the distance of the light source** at 15cm above the white tile. (Accept alternative distances as long as the number is logical and fixed.)
16. Attach a square piece of red filter such that it covers the entire circumference of the lamp shade by attaching the four sides of the square filter to the lamp shade using scotch tapes. In this way, a red lighting condition is obtained. Turn on the light. Remove the aluminium foil covering the capillary tubings. Repeat for the other colours.



17. Start the stop watch. Note and record the time taken for the blue colour to disappear.
18. Repeat the experiment from step 5 to 13 for the following wavelengths of light: ranging from blue, cyan, green, yellow, orange and red.
19. Repeat the experiment from steps 1 to 13 at least twice.
20. Repeat the entire experiment from steps 1 to 14 for the leaf extract from Var B.

Table showing the effectiveness of the different wavelengths of light in decolourising DCPIP for Species A.

Construct the same table for Species B

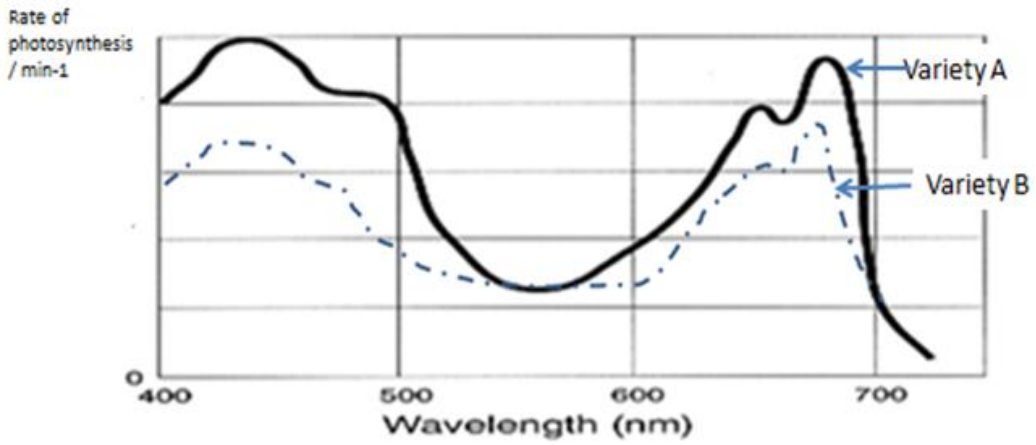
Wavelength of light/nm (Give actual value)	Time taken for DCPIP to decolourised/ min							Rate at which DCPIP decolourised/ min $^{-1}$
	Expt 1		Expt 2		Expt 3		Average	
	R1	R2	R1	R2	R1	R2		

625								
590								
565								
520								
435								
380								

Average time taken = [(R1+R2 of Expt 1)+ (R1+R2 of Expt 2)+ (R1+R2 of Expt 3)]/6

Rate at which DCPIP decolourised = 1/ average time taken

Graph showing the effectiveness of the different wavelengths of light in decolourising DCPIP.



[Total: 13]

3 During the question you will require access to a microscope and slide **K1**.

K1 is a slide of a stained transverse section through a leaf of a plant that grows in full sunlight and is adapted to relatively hot and bright conditions.

You are not expected to be familiar with this specimen.

(a) Use your microscope to observe the different tissues in the region of slide **K1** shown by the shaded area in Fig. 3.1.

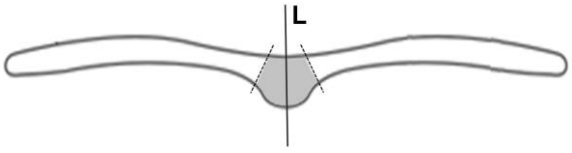


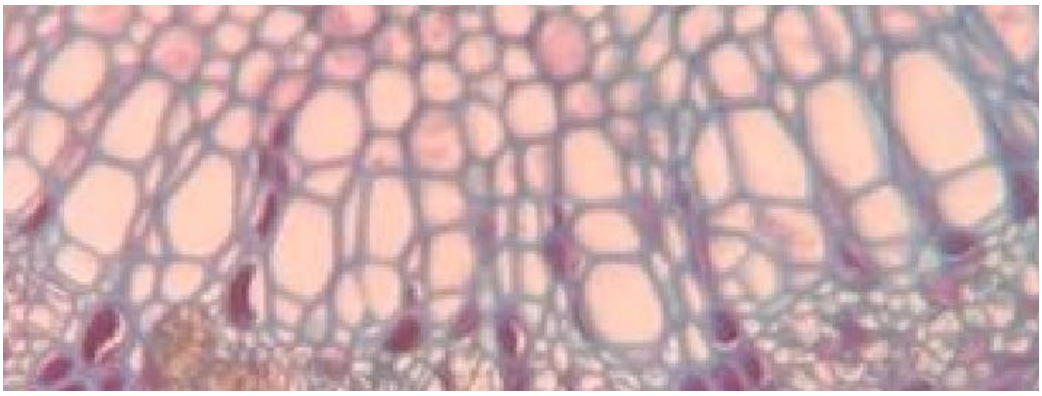
Fig. 3.1

(i) Select the largest cell in the region of slide **K1** shown by the shaded area in Fig. 3.1.

Use the space provided to make a large drawing of this cell **and** one other cell that this cell is touching.

Labels are **not** required.

1. **Two** cells drawn that are **joining/touching**, with **clean, continuous lines**, **that occupy at least half the space** provided.
2. Cells are of the **correct shape (angular)**, with (lignified) **wall of correct thickness/ proportion**.



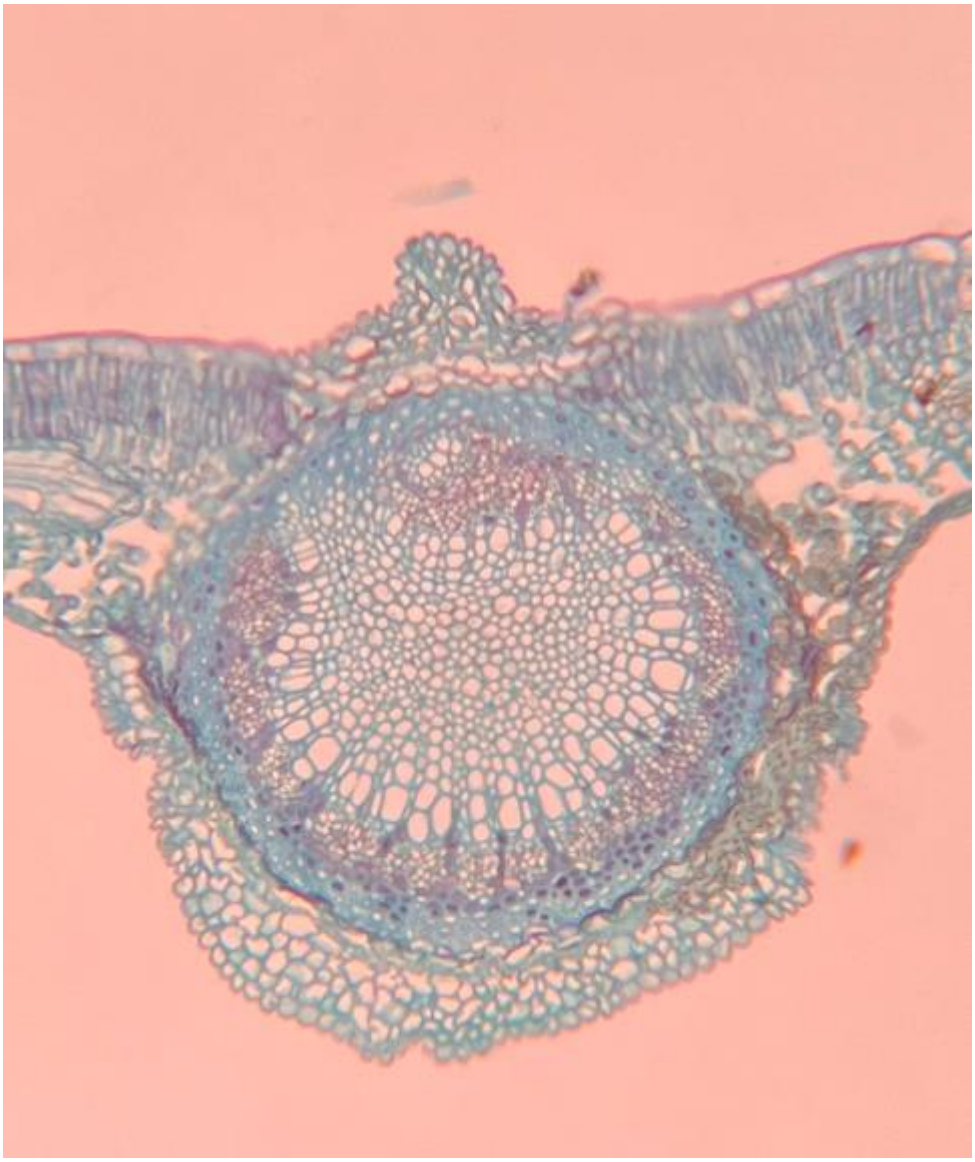
[2]

(ii) Use the space provided to draw a large plan drawing of the part of the leaf on slide **K1** shown by the shaded area in Fig. 3.1.

A plan drawing shows the arrangement of different tissues, including their correct shapes and proportions. No cells should be drawn.

Labels are **not** required.

1. No individual cells drawn. Drawing must occupy as **least half the space provided** vertically/ horizontally (and be appropriately large), and drawn with clear, continuous, smooth lines.
2. At least five distinct regions drawn – upper epidermis (with cuticle), bundle sheath, xylem, phloem, lower epidermis
3. correct shape and proportion of tissues
    - thin upper & lower epidermis
    - vascular bundle occupies at least ¾ of entire height of drawing
    - xylem 3-5 times thicker than phloem



[3]

(b) You are required to determine the thickness of the leaf on slide **K1**, along the line **L** labelled on Fig. 3.1 using low power (x4 objective lens).

(i) You are first required to calibrate the eyepiece graticule, using the clear plastic ruler provided.

Describe how to determine the length of one eyepiece graticule unit.

1. *(Place the ruler on the stage, focus under the x4 objective lens, and)* **align** the divisions/ markings of the **ruler** with those of the **eyepiece graticule**.
2. *(Ensure that both scales are aligned at one end/ at the zero mark)* and **count the number of eyepiece graticule units that correspond with a set number of ruler units**.
   (Knowing that one ruler unit is 1 mm, calculate the length of one eyepiece graticule unit) [2]

(ii) Carry out your method in **(b)(i)**, to determine the length of one eyepiece graticule unit.

Show your working.

Under the x4 objective lens,

40 eyepiece graticule units (A: 30 – 50 egus) = 1 ruler unit **[1]**
= 1 x 1 mm
= 1 mm

Thus, length of 1 eyepiece graticule unit = 1 mm / 40
= 0.025 mm **[1]** [2]

(iii) Using your answer to **(b)(ii)**, determine the thickness of the leaf on slide **K1**, along the line **L** labelled on Fig. 3.1.

Under x4 objective lens,

Thickness of leaf along line L = 28 eyepiece graticule units **[1]** *(A: 20-35)*
Length of 1 eyepiece graticule unit = 0.025 mm
Actual leaf thickness = 28 eyepiece graticule divisions x 0.025 mm
= 0.700 mm **[1]** [2]

(iv) Explain why the method in **(b)(i)** is likely to lead to an inaccurate determination of the length of one eyepiece graticule unit in **(b)(ii)**.

1. 1mm length measured by the ruler is of **low precision**/ when viewed under the x4 objective lens, **each scale marking of the ruler is already occupied by several eyepiece graticule units** [1]

(v) State two ways in which the accuracy of your answer in **(b)(iii)** can be improved.

1. using a **stage micrometer** instead of a ruler
2. measuring under a **higher power**/ **x10 objective lens**/ **x40 objective lens** [2]

(c) Fig. 3.2 is a photomicrograph of a stained transverse section through another species of leaf that also grows in a hot and bright environment.

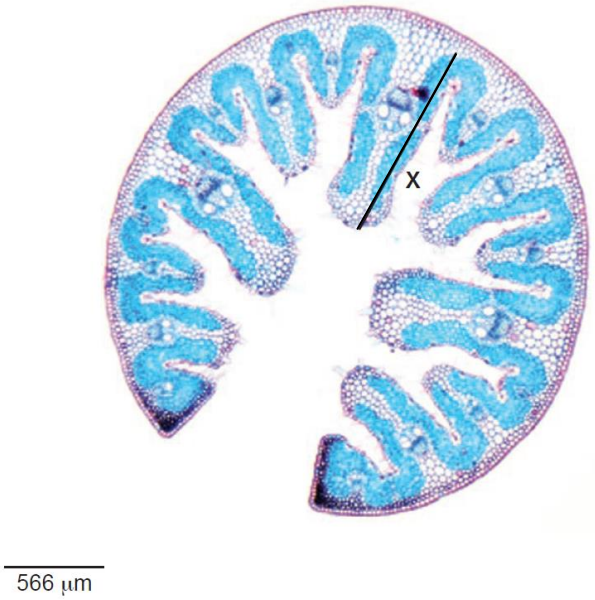


Fig. 3.2

(i) Calculate the actual length of the fold shown by line **X**, using the scale bar.

Show your working.

Magnification = 14 x 10^3 µm / 566 µm
= 24.7 x (3 s.f.) **[1]**

Actual length of fold = length of line X / magnification
= 28 (Accept: 28.5) x 10^3 µm / 24.7
= 1130 µm (3 s.f.)
= 1.13 mm (3 s.f.) **[1]** [2]

(ii) Complete Table 3.1 to identify different features of the leaf on Slide **K1** and in Fig. 3.2 that allow them to be adapted to hot and bright environments.
Explain your answer.

[K1]
1. feature: **thick cuticle** above upper epidermis
2. explanation: **prevents excessive water loss**

3. feature: **two layers** of **palisade mesophyll** cells/ **numerous chloroplasts** in **upper half** of leaf
4. explanation: **increase rate of photosynthesis** in bright environment

[Fig. 3.2]
5. feature: leaf blade is **curled up** such that stomata are hidden within/ **stomata are in sunken pits**
6. explanation: creates a region of **high humidity/ retains humidity** so that **water loss to leaf exterior is minimised** [4]

[Total: 20]