H

NATIONAL JUNIOR COLLEGE, SINGAPORE
Senior High 2
Preliminary Examination
Higher 2

CANDIDATE NAME			
BIOLOGY CLASS	2bi2_____	REGISTRATION NUMBER	

Biology

9744/04

Paper 4 Practical

17 August 2023

2 hours 30 minutes

READ THESE INSTRUCTIONS FIRST

Write your name, Biology class, and registration number on all the work you hand in.
Give details of the practical shift and laboratory in the boxes provided.
Write in dark blue or black pen on both sides of the paper.
You may use a soft pencil for any diagrams, graphs.
Do not use staples, paper clips, highlighters, 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 workings 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 the brackets [ ] at the end of each question or part of question.

Shift			
1	2	3	
Laboratory			
BI23	BI24	CM43	CM44

For Examiner's Use	
1	19
2	17
3	19
Total	55

This document consists of **18** printed pages and **2** blank pages.

Answer **all** questions.

1 Baker's yeast, *Saccharomyces cerevisiae*, converts sugars to ethanol and carbon dioxide under anaerobic conditions.

You will investigate the effects of different concentrations of ethanol on the rate of respiration in yeast.

(a) Describe and explain the expected effect of increasing ethanol concentration on the rate of respiration in yeast.

As the **concentration of ethanol increases**, the **rate of respiration decreases**;
Ethanol is organic and thus dissolves and **disrupts phospholipid bilayer**;
This **destroys the integrity of membrane** essential **for respiration**, namely the inner mitochondrial membrane;
Membrane proteins such as electron carrier and ATP synthase on the inner mitochondrial membrane become **non-functional**;
Enzymes are also denatured by the high ethanol concentration by the disruption of hydrogen bonding or hydrophobic interactions;
High concentration of ethanol **inhibits anaerobic respiration** / leads to **end product inhibition** thus there is a decrease in CO2 released;

R: Change in pH, decrease in frequency of effective collision (ethanol is not within the cell)

[4]

You will set up the apparatus as shown in Fig. 1.1.

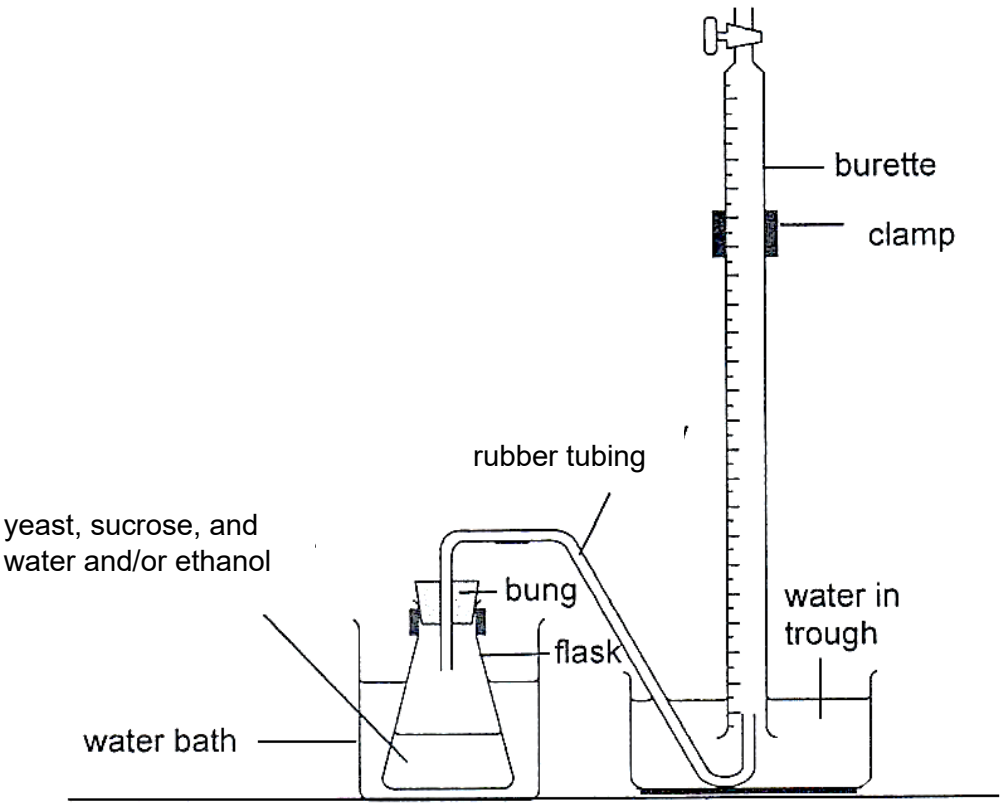


Fig. 1.1

You are provided with:

- 5.0 g dried yeast **Y**, in a container labelled **Y**
- 10.0 g sucrose **S**, in a container labelled **S**
- 120 cm^3 5.0 % ethanol **E1**, in a container labelled **E1**
- 120 cm^3 10.0 % ethanol **E2**, in a container labelled **E2**
- 200 cm^3 distilled water **W**, in a beaker labelled **W**

E1 and E2 contain ethanol, which is harmful and flammable. Suitable eye protection should be worn. The lid on the plastic vial should be kept on, when not in use.

Read steps **1-11**.

Proceed as follows.

1 Set up a water bath and maintain it at about 37 °C.

2 Measure 100 cm^3 of **W** and pour it into a conical flask.

3 Weigh 1.0 g of **Y** and 2.0 g of **S** and add them to the conical flask. Shake to mix the content.

4 Incubate the conical flask in the water bath for 5 minutes.

5 Fill the burette with water. Invert the burette and clamp it on a retort stand, as shown in Fig 1.1.

6 Attach the rubber bung (with the rubber tubing) to the conical flask.

7 Insert the rubber tubing through the open end of the burette. Ensure that the apparatus is set up as shown in Fig. 1.1.

8 After 5 minutes of incubation, note the initial volume of water in the burette.

9 Measure the volume of carbon dioxide given off for 8 minutes by the amount of water displaced.

10 Repeat steps **1**-**9** using 100 cm^3 of **E1**.

11 Repeat steps **1**-**9** using 100 cm^3 of **E2**.

(b) Record your results in an appropriate table.

Concentration of ethanol / %	Volume of carbon dioxide given off in 8 minutes / cm^3
0	10.0
5	2.7
10	0.1

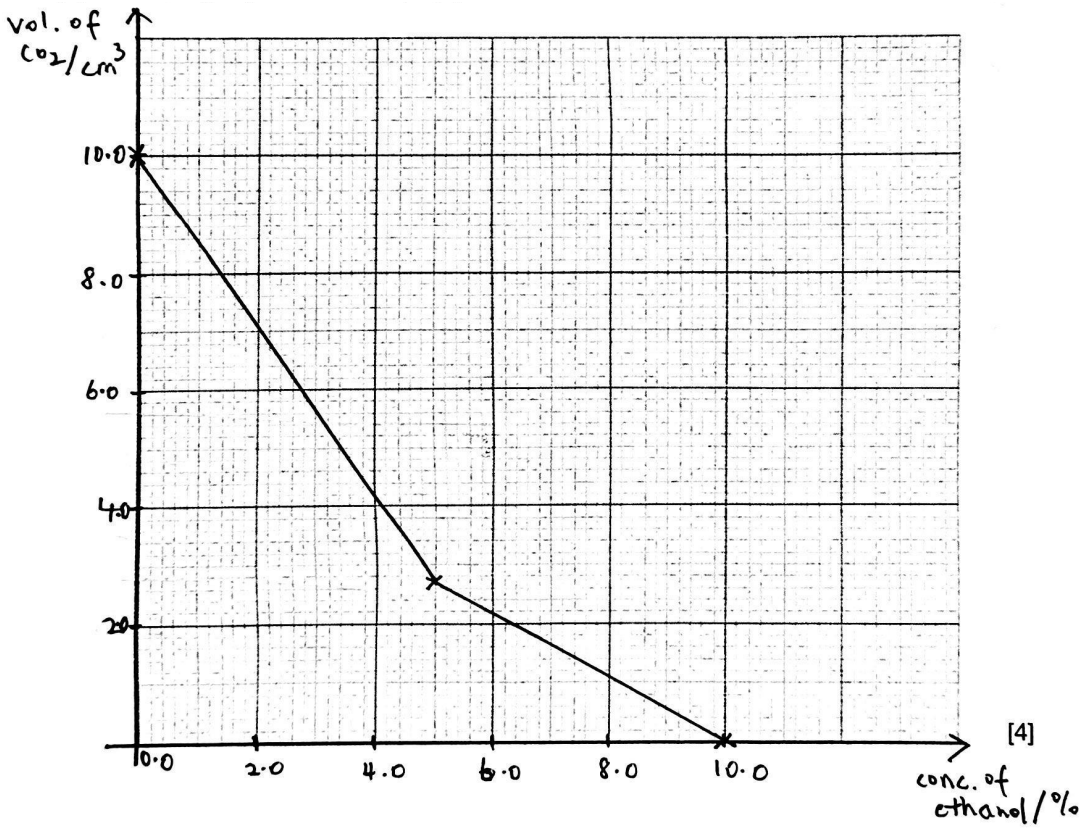
H – Headings with units;

P – Precision of data (Vol. of CO_2 at 1 or 2 d.p.);

T – Trend of data (Decrease in CO_2 with increase in ethanol concentration);

[3]

(c) Use the grid provided to display your results.



[4]

G – Graph: Dot-to-dot plot (**R**: Histogram);

A – Axes with labels and units;

P – Points are all plotted accurately;

S – Scale of the graph should at least 2/3 of space provided;

Examiner's comments: A number of candidates did not recognise that this experiment is to investigate the effects of different concentrations of ethanol on the rate of respiration in yeast. As such, water should be represented as 0% with respect to the concentration of ethanol. The graph is not a histogram depicting categorical or discrete data. A few candidates drew best fit line / curve instead. There are also some students who lost marks on scale, plots or axes with units.

(d) Without quoting numerical values, explain how this experiment allows you to monitor respiration in yeast.

In **link reaction** and **Kreb's cycle** of aerobic respiration / **alcoholic fermentation** of anaerobic respiration, **CO_2 is released**;
CO_2 produced from the conical flask mixture is passed through the rubber tubing and collected in the burette;
The increase in CO_2 within the burette leads to **water being displaced**, thereby allowing monitoring of respiration over time;
Therefore, **increase in volume of CO_2** produced indicates an **increase in rate of respiration**;

[3]

(e) Describe **two** main sources of error in the procedure and discuss how they may reduce the confidence in your results recorded in **(b)**.

The water bath temperature is **difficult to maintain at 37°C**;
As respiration is an **enzyme**-catalysed reaction and enzyme is sensitive to temperature, the **varying temperature may affect the rate of reaction**. The rate of reaction is not constant and thus the final volume of CO_2 collection is not accurate;
The release of CO_2 is measured by the displacement of water in the burette, however, this is not accurate as **some CO_2 may dissolve in water**;
Thus, the reading taken is **not an accurate reading of the actual volume of gas produced**;

AVP (precision of scale, wind affecting reading of scale)

R: Under-estimation of CO_2 produced due to incubation time; Air bubbles have different volumes; measuring cylinder is not a precise instrument; human errors such as uneven mixing of sucrose and yeast in solution or escape of CO_2 from the conical flask with rubber bung;

Examiner's comments: When describing effects of temperature affecting rate of reaction, some wrote about increase in temperature which is not possible as there is only heat loss to the environment. Some candidates also included improvement which is not a requirement of the question. In addition, human error should be avoided.

[4]

(f) Suggest a negative control for this experiment to prove the action of yeast in converting sugars to ethanol and carbon dioxide.

Replace Y with **boiled yeast** where the enzymes are denatured.

R: Use of distilled water, powder / sand / particles

[1]

[Total: 19]

2 Fig. 2.1 shows *Elodea canadensis* (Canadian pondweed) which is an aquatic plant commonly used in aquarium tanks to help control algae and keep the water clear.

Fig. 2.1

Fig. 2.2 shows how *Elodea* form dense mats on the surface of water in an aquarium tank.

Fig. 2.2

Studies suggest that one mechanism by which *Elodea* inhibits algae is via competing for light for photosynthesis.

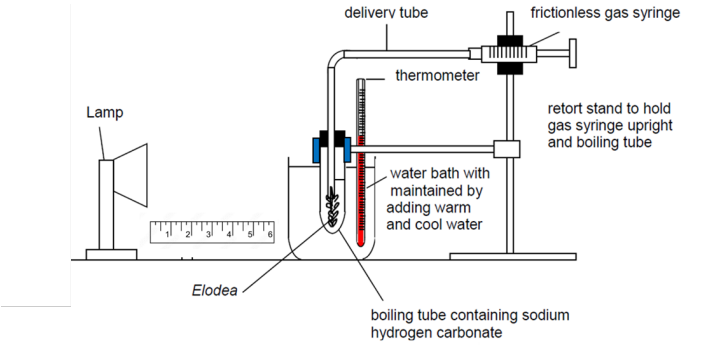
(a) (i) Design an experiment to show that the rate of photosynthesis in *Elodea* is dependent on light intensity.

In your plan you must use:

- a room which can be made dark
- table lamp
- *Elodea* (Canadian pondweed)
- ruler
- scalpel
- sodium hydrogen carbonate powder
- weighing balance
- measuring cylinder
- distilled water
- boiling tube
- delivery tube
- gas syringe
- rubber bung
- thermometer
- stopwatch
- beaker
- retort stand

Your plan should:

- identify the dependent variable and independent variable
- identify variables you will need to control
- have a clear and helpful structure so that the method described could be repeated by anyone reading it
- be illustrated by relevant diagrams, if necessary
- include details to ensure that results are accurate and repeatable as possible
- indicate how results will be recorded and analysed
- use the correct technical and scientific terms
- include reference to safety measure to minimise any risks associated with the proposed experiment

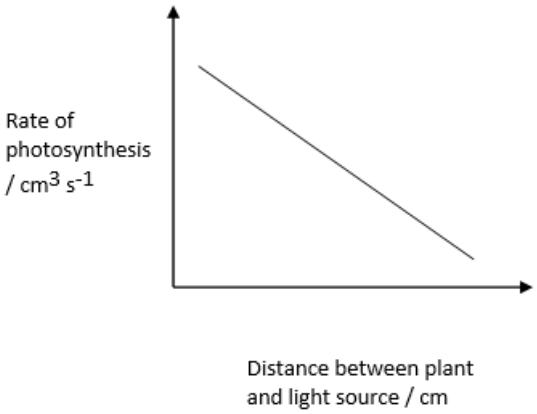
Independent variable = light intensity, derived from distance between Elodea and lamp; Dependent variable = Rate of photosynthesis calculated by volume of oxygen evolved per second / cm3 s-1;	State IV and DV;
 Labelled diagram of experimental setup Elodea with sodium hydrogen carbonate in boiling tube in temperature monitored water bath Gas syringe connected correctly for measurement of gas volume using rubber bung and delivery tube Use of ruler and table lamp	**MAX 4 for procedure + Diagram** Labelled diagram of experimental setup;
1. Weigh 2 g of sodium hydrogen carbonate using a weighing balance, and add to 40 cm3 of distilled water in a boiling tube measured using measuring cylinder. 2. Cut the stem of piece of *Elodea* to 5 cm length with a sharp knife 3. Place cut stem into boiling tube 4. Place tube into a water bath at 30°C maintain this temperature by adding warm/cool water, monitor with thermometer. 5. Use a delivery tube to connect boiling tube to a gas syringe. 6. Darken the room. Place the table lamp 10 cm from the *Elodea* measured by ruler. 7. Allow the plant to acclimatize to light intensity and temperature of water bath for 3 minutes till rate of bubbling has stabilized 8. Record initial value on syringe 9. Collect gas produced over 5 minutes and record final value on syringe. Use stopwatch to time. 10. Calculate dependent variable, which is rate of photosynthesis calculated by rate of oxygen produced by dividing volume of oxygen by time 11. Repeat the above procedure for increasing distances between the table lamp and Elodea, such as 20, 30, 40 and 50 cm 12. Perform triplicates for each distance for reliability 13. Repeat experiments twice with fresh reagents for reproducibility	Suitable range of light intensities (at least 5 distances from the light source); State range. Eg.10 cm to 20 cm; Describe procedure to monitor DV over a set time period; Other variables to be kept constant - amount of sodium hydrogen carbonate used/ CO_2 concentration in water; Length of stem cut; Monitoring of constant temperature

<table>
<tr>
<td>14. Set up control experiment with a new plant specimen. All conditions remain the same except there is no light source. This shows oxygen released is due to plant photosynthesizing in the light (purpose).</td>
<td>using thermometer; [Max 2]

Time set aside for plant to acclimatise / equilibration procedure;

3 replicates and 3 repeats;

Negative control;</td>
</tr>
<tr>
<td>
<table>
<tr><th rowspan="2">Distance between plant and light source / cm</th><th colspan="4">Volume of oxygen gas evolved in 5 min / cm^3</th><th rowspan="2">Rate of photosynthesis (Volume of oxygen gas evolved per min) / $cm^3\ s^{-1}$</th></tr>
<tr><th>Replicate 1</th><th>Replicate 2</th><th>Replicate 3</th><th>Mean</th></tr>
<tr><td>10</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>20</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>30</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>40</td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>50</td><td></td><td></td><td></td><td></td><td></td></tr>
</table>
</td>
<td>Present table for recording results with IV/units, DV/units in appropriate column and row;

Table with replicates and rate in terms of volume of oxygen gas evolved per unit time;</td>
</tr>
<tr>
<td> 
</td>
<td>Present graph for charting relationship between DV/units and IV/units;</td>
</tr>
<tr>
<td>Care with sharp knife to avoid cuts when trimming stems
Care when handling the lamp to prevent scalding with bulb</td>
<td>State significant hazard and</td>
</tr>
</table>

Dry hands when operating the lamp to prevent electric shock	appropriate precaution;

[8]

(ii) Explain how the growth rate of organisms like plants and algae is correlated with the rate of photosynthesis.

Higher photosynthesis rates enable more glucose utilized by the plant;
in respiration for ATP/energy;
Higher rate of ATP production from respiration allows for greater protein synthesis/cell division rate for growth

[2]

(iii) Besides competing for light, suggest one other mechanism by which *Elodea* controls algae growth in aquarium tanks.
competes with algae for essential nutrient (Elodea limits their availability to algae, thereby reducing their growth potential);
releases oxygen during photosynthesis, which can inhibit the growth of algae;
AVP

[1]

(b) An aquarium company has claimed that their biological fertiliser 'Swamp Thing' is superior to current market products in enhancing the growth of aquatic plants.

An NJC student set out to test this claim. He carried out an experiment to measure the mass of two groups of *Elodea* after a 10-weeks period of growth. One group was grown

in tanks that were treated with 'Swamp Thing'. The other group was grown with 'Groot', a well-established fertiliser developed by another reputable Aquarium company. All other variables were controlled.

His results are enclosed in Table 2.1

Table 2.1

sample number	mass of *Elodea* / g	
	'Swamp Thing'	'Groot'
1	1.76	0.49
2	1.45	0.85
3	1.03	1.00
4	1.53	1.54
5	2.34	1.01
6	1.96	0.75
7	1.76	2.11
8	1.27	0.92
mean ($\bar{x}\bar{x}$)	1.64	1.08

(i) Complete Table 2.1 by calculating the mean for the mass of both *Elodea* groups.

[2]

(ii) A t-test was carried out to see if *Elodea* grown with 'Swamp Thing' was significantly greater in mass compared to those grown with 'Groot'.

Suggest a null hypothesis for this statistical test.

Mass between Elodea grown with Swap Thing and that grown with Groot is similar;

/Mass between Elodea grown with Swap Thing and that grown with Groot is the same. Any difference is due to chance;

There is no significant difference in mass between *Elodea* grown with Swap Thing and that grown with Groot;

--- [1]

(iii) Table 2.2 shows the critical values for t at different probabilities and degrees of freedom for one-tailed and two-tailed tests.

Table 2.2

degrees of freedom	probability, *p*, for one-tailed test			
	0.25	0.05	0.025	0.005
	probability, *p*, for two-tailed test			
	0.5	0.1	0.05	0.01
1	1.00	6.31	12.71	63.66
2	0.82	2.92	4.30	9.92
3	0.76	2.35	3.18	5.84
4	0.74	2.13	2.78	4.60
5	0.73	2.02	2.57	4.03
6	0.72	1.94	2.45	3.71
7	0.71	1.89	2.36	3.50
8	0.71	1.86	2.31	3.36
9	0.70	1.83	2.26	3.25
10	0.70	1.81	2.23	3.17
11	0.70	1.80	2.20	3.11
12	0.70	1.78	2.18	3.05
13	0.69	1.77	2.16	3.01
14	0.69	1.76	2.14	2.98
15	0.69	1.75	2.13	2.95
16	0.69	1.75	2.12	2.92
17	0.69	1.74	2.11	2.90
18	0.69	1.73	2.10	2.88
19	0.69	1.73	2.09	2.86
20	0.69	1.72	2.09	2.85

The calculated t-value was determined to be 3.04.

Using the t-distribution table above, explain what conclusions can be drawn from the calculated t-value.

At df = 14;

calculated t-value is bigger than the critical t-value of 1.76 at 0.05 significance level/ The probability that the difference in mass between Elodea grown with Swap Thing and that grown with Groot due to chance is less than 0.05 (cut-off);
Elodea grown with 'Swap Thing' was significantly greater in mass compared to those grown with 'Groot';

--- [3]

[Total: 17]

3 For this question, you will require access to a microscope, slide **S1** and specimen **S2**.

You will observe and compare the structures of the stem of two different climbing plants. A climbing plant is a plant that attaches itself to a structure, such as a fence, as it grows.

Cucurbita is a genus of herbaceous vines consisting of squash, pumpkin, gourd, etc. It is a climbing plant that can produce stems up to 5 metres long (Fig. 3.1).

Fig. 3.1

S1 is a slide containing both a stained transverse section and a stained longitudinal section of a stem of *Cucurbita*.

You are not expected to be familiar with these specimens.

(a) Use the microscope to observe the different tissues in the transverse section of the stem on **S1**.

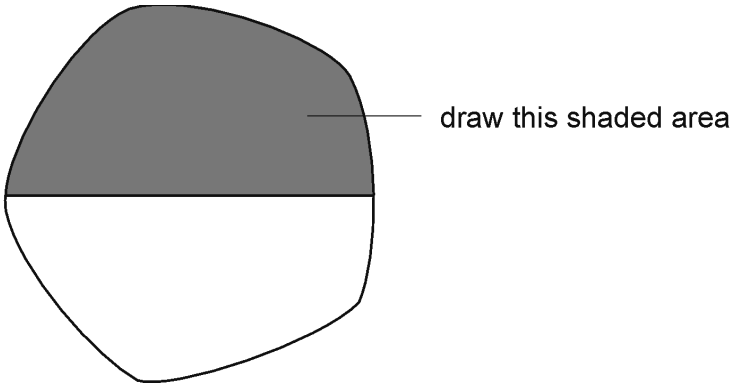


Fig. 3.2

Draw a large plan diagram of half of the transverse section as shown in Fig. 3.2.

A plan diagram shows the arrangement of different tissues. Your drawing should show the correct shapes and proportions of different tissues.

Outline all the vascular bundles but do not include any details of them. Your drawing should accurately show the numbers of the bundles, their sizes and positions.

No cells should be drawn.

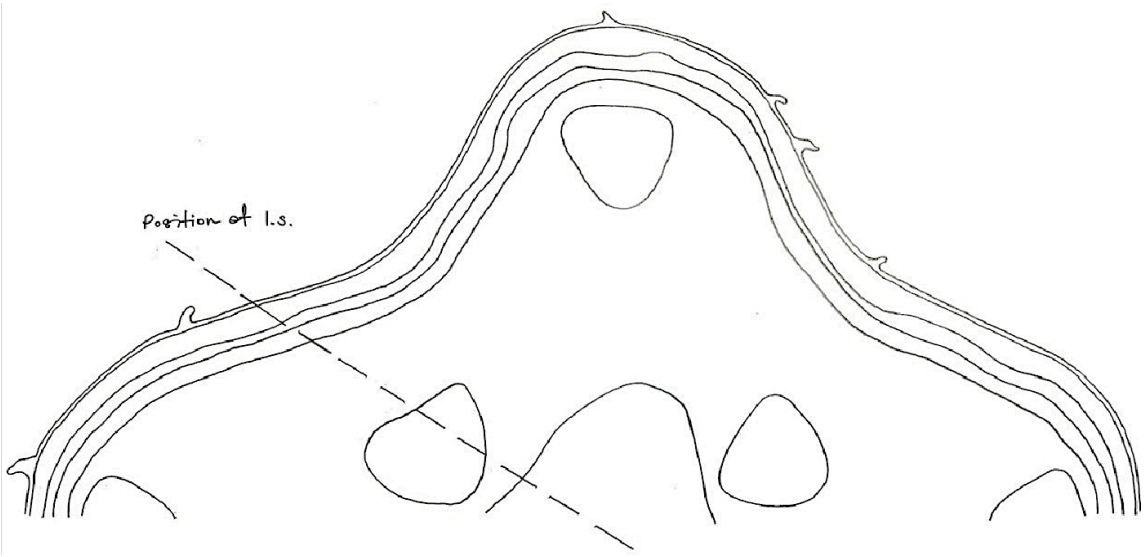
C – clear and continuous lines, no shading

S – fill up ¾ of the space (at least 11cm horizontally), drawing of half of the transverse section, correct overall shape of stem and pith cavity (irregular star shape)

P – correct proportion of layers of tissues (follow ratios in blue, epidermis is about 1/3 of the outer cortex layer D)

D – correct proportion and positions of vascular bundles (rounded conical shape vascular bundles alternate outside and inside corresponding to the corners of the star shape, vascular bundles inside are larger than outside, follow ratios in red)

L – correct number of layers (at least 5 layers: epidermis, outer cortex layer, red layer, ground tissue, pith cavity)



[5]

(b) The distribution of the different tissues in the vascular bundle in the stem of *Cucurbita* is unusual in that there are two regions of phloem in each vascular bundle in the stem of *Cucurbita*. The distribution is shown in Fig. 3.3.

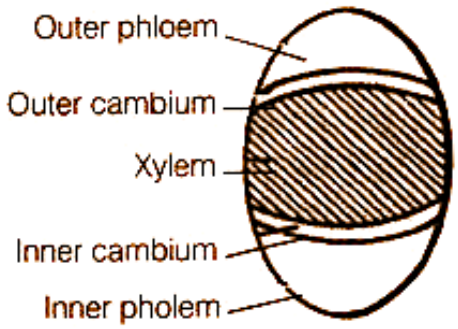


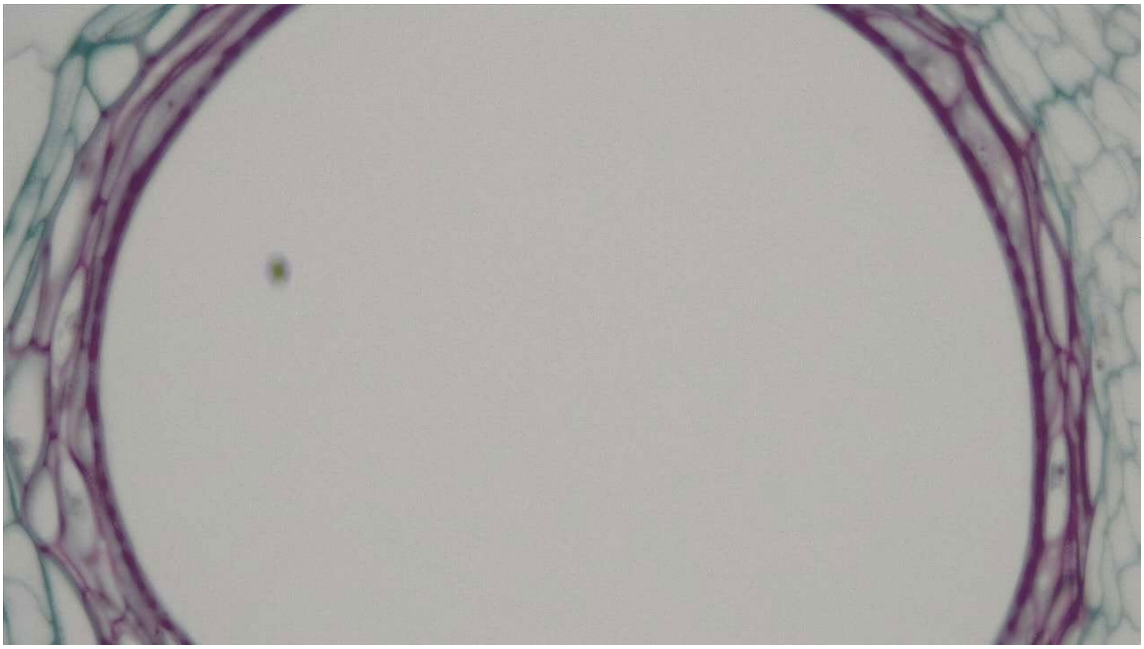
Fig. 3.3

Examine the transverse section of the stem on **S1** using high power objective of your microscope. Find a vascular bundle and observe the distribution of the different tissues in the vascular bundle.

(i) Select a group of cells consisting of one large xylem vessel and one layer of adjacent cells touching the large xylem vessel.

Make a large drawing of this cluster of cells.

Labels are **not** required.

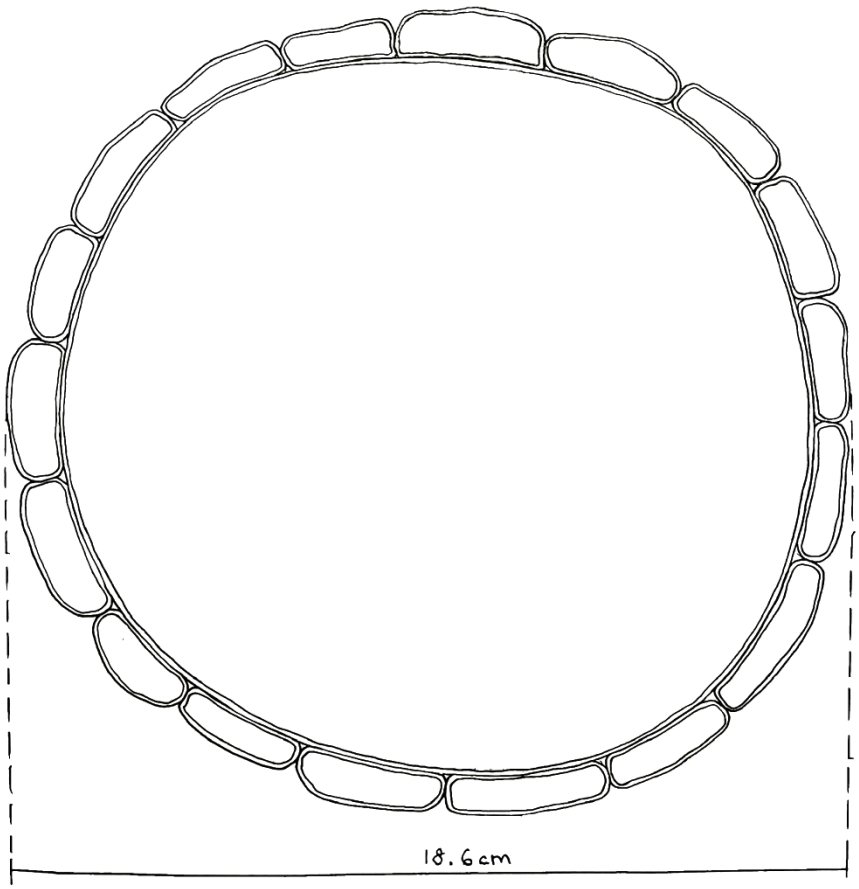


C – clear and continuous lines, no shading

S – fill up ¾ of the space (height at least 8cm or length at least 10cm)

P – correct proportion of shape and size of cells (one large xylem vessel at least 10 times larger than surrounding cells)

D – cell wall touching neighbouring cells, a layer of at least 10 small cells surrounding the large xylem vessel, no nucleus



[4]

Examiner's comments:

Some students were confused between transverse (cross-section) vs longitudinal (cut along the length) sections. Many students were penalised on proportionality of layers of tissues and size of cells. Common errors which could have been avoided are unclear/hairy lines and small drawing occupying less than ¾ of the space.

(ii) Calibrate your eyepiece graticule at high power (x40 objective lens), using a stage micrometer.

Calculate the absolute dimensions of one eyepiece graticule unit based on this calibration.

Show your working.

40 eyepiece graticule units (accept 35 – 45) = 1 stage graticule unit = 0.1mm

1 eyepiece graticule unit = 0.1 / 40 = 0.0025 mm ;

= 2.5 µm (unit conversion) ;

1 eyepiece graticule unit = ……µm [2]

Examiner's comments:

Most students were able to do the calibration and calculation. However, some memorised the final value and did the workings backwards e.g. 100 eyepiece units = 2.5 stage units, such workings are not accepted as you should not be using a fraction of the graticule unit in your calibration.

(iii) Using the calibrated eyepiece graticule in **(b)(ii)**, measure and calculate the diameter of the large xylem vessel drawn in **(b)(i)**.

Diameter of large xylem vessel in eyepiece units = **a** (accept: 30 – 120)

Absolute dimensions of the diameter of the large xylem vessel = **a** x **(ii)**

Reject smaller size (<30 eyepiece units) as qn ask for large xylem vessel.

diameter of large xylem vessel = ……µm [1]

(iv) Calculate the magnification of the large xylem vessel drawn in **(b)(i)**.

Diameter of drawing of large xylem vessel = **b**

Magnification = **b** / **(iii)**

Examiner's comments: A few students made errors in the conversion of units.

magnification = x …… [1]

Examine the longitudinal section of the stem on **S1**, using your microscope.

(c) **(i)** On your drawing in **(a)**, indicate one possible position at which the longitudinal section could have been cut. Label this "*position of l.s.*".
[1]

(ii) Give a reason for your decision in **(c)(i)** based on your observation of both the transverse and longitudinal sections.

Longitudinal section has **a large space** (pith cavity) **in the middle** with **a vascular bundle (stained red) on each side** of the space; [1]

Examiner's comments: Part (c) is poorly done. Many students can't seem to identify the vascular bundles / xylem in the longitudinal section, and claim that it is absent. The xylem is stained pink and so students can identify by the colour or by the structure of spiral/pitted vessels. Also, many students seem to only have observed one side of the longitudinal section and did not realise that the central cavity separates two sides of the longitudinal section. Some students wrongly indicated the position at a single point instead of a line across the transverse section, while others labelled in part (b) instead of part (a).

(iii) Pith is the soft spongy tissue in the central region of vascular plant stems. In *Cucurbita*, the central part of the pith disintegrates to produce a cavity, as seen from the large gap in the middle of the longitudinal section on **S1**. This cavity is called the pith cavity, which is an important adaptation in some climbing plants.

Suggest one benefit to a climbing plant for having a hollow stem instead of a solid stem.

Ref to: allows stem to be **flexible** to wind around and cling onto support structures without breaking;

Ref to: **reduce mass/weight** of plant so that it is easier to support as it climbs upwards; [1]

Examiner's comments: This question is generally well done. However, some students mistook the cavity as a xylem vessel and wrote its function in transport, which does not serve a specify benefit for climbing plants.

(d) *Epipremnum aureum*, commonly known as money plant, is another type of climbing plant.

Proceed as follows to prepare a transverse section of the stem of **S2**.

1 Use a sharp penknife to cut a few very thin (< 1mm thick) transverse section slices of the stem of **S2**. **Be careful when handling the sharp penknife.**

2 Place one of the thinnest slices in the middle of a clean slide.

3 Add 1-2 drops of water and then apply a cover slip.

The stem of *Cucurbita* (**S1**) is hollow while the stem of **S2** is often, but not always, expected to be completely filled with cells.

Identify three **other** observable structural differences between the stem of *Cucurbita* (**S1**) and *Epipremnum aureum* (**S2**).

Record these **three** observable differences in Table 3.1.

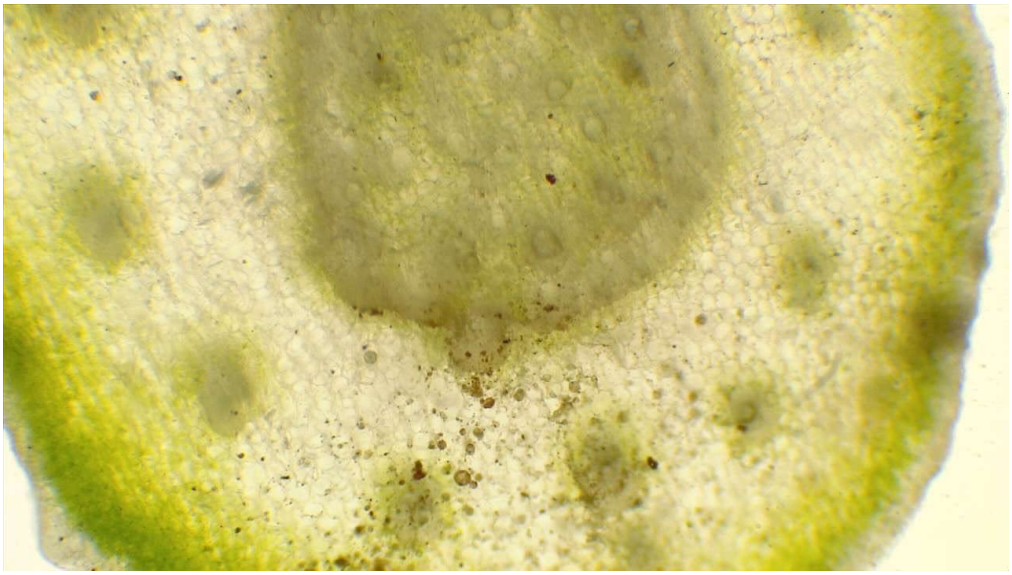


Table 3.1

feature	*Cucurbita* (S1)	*Epipremnum aureum* (S2)
Shape of stem	Star-shaped / five-sided / irregular shape	Circular / round shape (reject spherical)
Arrangement of vascular bundles	Arranged in two radial layers, inner and outer rings	Arranged in one radial layer
Relative size of vascular bundles	Larger	Smaller
Variability in size of vascular bundles	Inner ring of vascular bundles are larger than outer ring	Uniform
Layers of tissues	More	Fewer
Size/Thickness of stem	Larger	Smaller
Hair-like protrusions on epidermis	Present	Absent

Accept number of vascular bundles ONLY IF numbers are provided (reject more vs less as it could be either depending on size of the stem – vascular bundles are countable hence must be counted for comparison)

Examiner's comments:

This part is poorly done as many students compared features which are not clearly visible in the sample or are entirely absent. E.g. reference to chlorophyll, chloroplasts, and details in the vascular bundles are penalised as they are not visible under the light microscope in at least one of the sample.

Reject ref to pith / central tissue of stem / air spaces as question asked for "other" observable differences.

[3]

[Total: 19]

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