HWA CHONG INSTITUTION
JC2 Preliminary Examinations
Higher 2

CANDIDATE NAME		**CT GROUP**	**22S7 __**
CENTRE NUMBER		**INDEX NUMBER**	

BIOLOGY

9744/04

Paper 4 Practical

28 August 2023

Candidates answer on the Question Paper.

2 hours 30 minutes

No Additional materials are required.

INSTRUCTIONS TO CANDIDATES

There are **two** question booklets (**I** and **II**) to this paper. Write your **name**, **CT group**, **Centre number** and **index number** in the spaces provided at the top of this cover page and on the lines provided at the top of the cover page of Booklet **II**.

Answer **all** questions in the spaces provided on the question paper.

Shift
Laboratory

INFORMATION FOR CANDIDATES

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.

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

You are reminded of the need for good English and clear presentation in your answers.

For Examiners' Use	
1	**/ 30**
2	**/ 25**
Total	**/ 55**

This document consists of **23** printed pages and **1** blank page.

QUESTION 1

Part A

The process of photosynthesis traps light using a range of pigments bound to chloroplast membranes. The development of these pigments and chloroplasts in young plants is influenced by their growth conditions.

In this experiment you are required to investigate the pigments and chloroplasts in two sets of young plants, **A** and **B**, grown in different conditions.

You must keep the two types of leaves separate throughout the investigation.

Observe and make brief notes of any differences between a leaf from plant **A** and a leaf from plant **B**.

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Procedure 1 (To be attempted when you receive the light microscope)

1 Cut the leaves from specimen **A** into four pieces, each about 3 cm by 3 cm. Set aside two pieces of the leaves from specimen **A** for **Procedure 2**.

2 Place two pieces of the leaves from specimen **A** in a mortar. Add 4 cm^3 distilled water and grind with a pestle for 1 minute.

3 Filter the suspension through the muslin cloth provided, onto a Petri dish.

4 Use the Pasteur pipette provided to put a drop of filtrate onto a microscope slide and cover with a coverslip. Observe under $\times$100 and $\times$400 magnifications.

5 Repeat steps **1 - 4** using the leaves from specimen **B**.

(a) State two differences between the chloroplasts from specimen **A** and specimen **B**.

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..... [2]

Procedure 2

6 Add 5 cm^3 of ethanol into each of the two tubes labelled **X** provided. Label the tubes **A** and **B** respectively with a marker.

7 Put the cut pieces of leaves from specimen **A** and specimen **B** on separate clean paper towels. Remove any thick veins from the leaves.

8 Tear the cut pieces of leaves from specimen **A** into small pieces and place them into the corresponding tube of ethanol solution labelled **A**. Use a glass rod to push the leaves to the bottom of the tube. Place a stopper into the mouth of the tube.

9 Repeat step **8** using the cut pieces of leaves from specimen **B** and the corresponding tube of ethanol solution labelled **B**.

10 Place both tubes into a beaker.

11 Pour sufficient water from the 90 °C water bath into the beaker to reach the top of the extract in each tube. Incubate the tubes in the 90 °C water bath in the beaker for 10 minutes.
Caution: Be careful when working with hot water.
Continue with step 23 on pages 6 to 7 during this time.

12 Remove the tubes from the water bath carefully and allow to cool.

13 Remove the two strips of chromatography paper from the plastic bag and place on a clean paper towel.

14 Draw a pencil line 25 mm from one end of each piece of the chromatography paper. Label lightly in pencil one strip to use for extract **A** and the other to use for extract **B**.
Caution: Do not draw the line with ink pen.

15 For each extract, use a micropipette tip to pick up a length of about 5 mm of the extract by using the tip to touch the surface of the extract. Apply a spot in the centre of the pencil line by touching it briefly against the centre of the pencil line. Allow it to dry and then apply another spot on top of the first spot.

16 Continue to apply spots until all the extract at the tip has been applied. Pick up another 5 mm of the extract and repeat step **15**.

17 Fold the top of the paper 5 mm from the other end so that it is ready to be pinned to the underside of a boiling tube stopper.

18 Remove the stoppers from the top of the boiling tubes containing solvent. Pin the paper on the stopper and insert it into the boiling tube until the bottom of the paper just touches the solvent as shown in Fig.1.1. Make sure the paper does not touch the sides of the boiling tube.

Caution: The solvent is flammable. Do not remove the stoppers from the boiling tubes if there are any lighted Bunsen burners or other flames in the room. Do not pour the solvent into the sink at the end of the experiment.

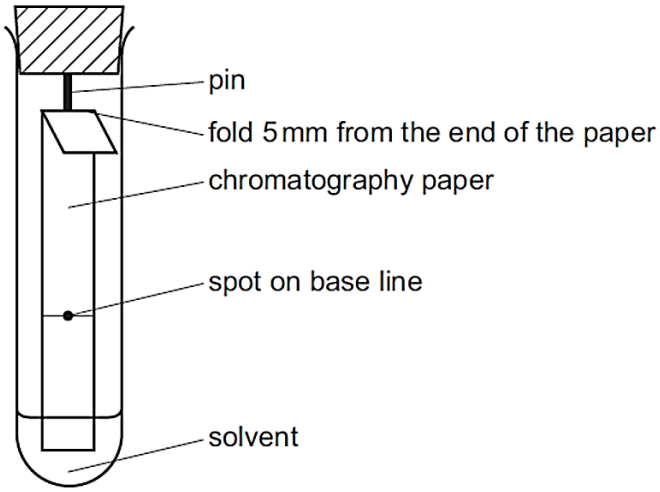

Fig. 1.1

19 Leave the boiling tubes in a rack until the solvent front is almost at the fold. Do not move or shake the tubes during this time.

Continue with step 23 on pages 6 to 7 while this is happening.

20 Remove the strips of paper from the boiling tubes. Mark in pencil the position of the solvent front and the front edge of each pigment immediately, since the colour of the pigments fades quickly. Leave to dry on a clean paper towel.

(b) Prepare the space below to record your results in an appropriate format so that you can compare extract **A** with extract **B**. Include the R_f value for each pigment calculated as instructed in step **21**.

[4]

21 Note the colour for each pigment you can see on each chromatogram. Work out their R_f values using the formula $R_f = \frac{distance\ moved\ by\ pigment\ from\ base\ line}{distance\ moved\ by\ solvent\ from\ base\ line}$

You should measure to the front edge of each pigment.
Record your results in the space you have prepared above.

22 Record in pencil on each chromatogram what you measured.

(c) Label your chromatograms **A** and **B** and paste them in the space below using the sticky tapes provided. [1]

(d)(i) Other than lack of replicates, suggest **one** source of error in this procedure and explain how it affects your results.

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..... [2]

(ii) Suggest an improvement that can be made to eliminate the error.

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..... [1]

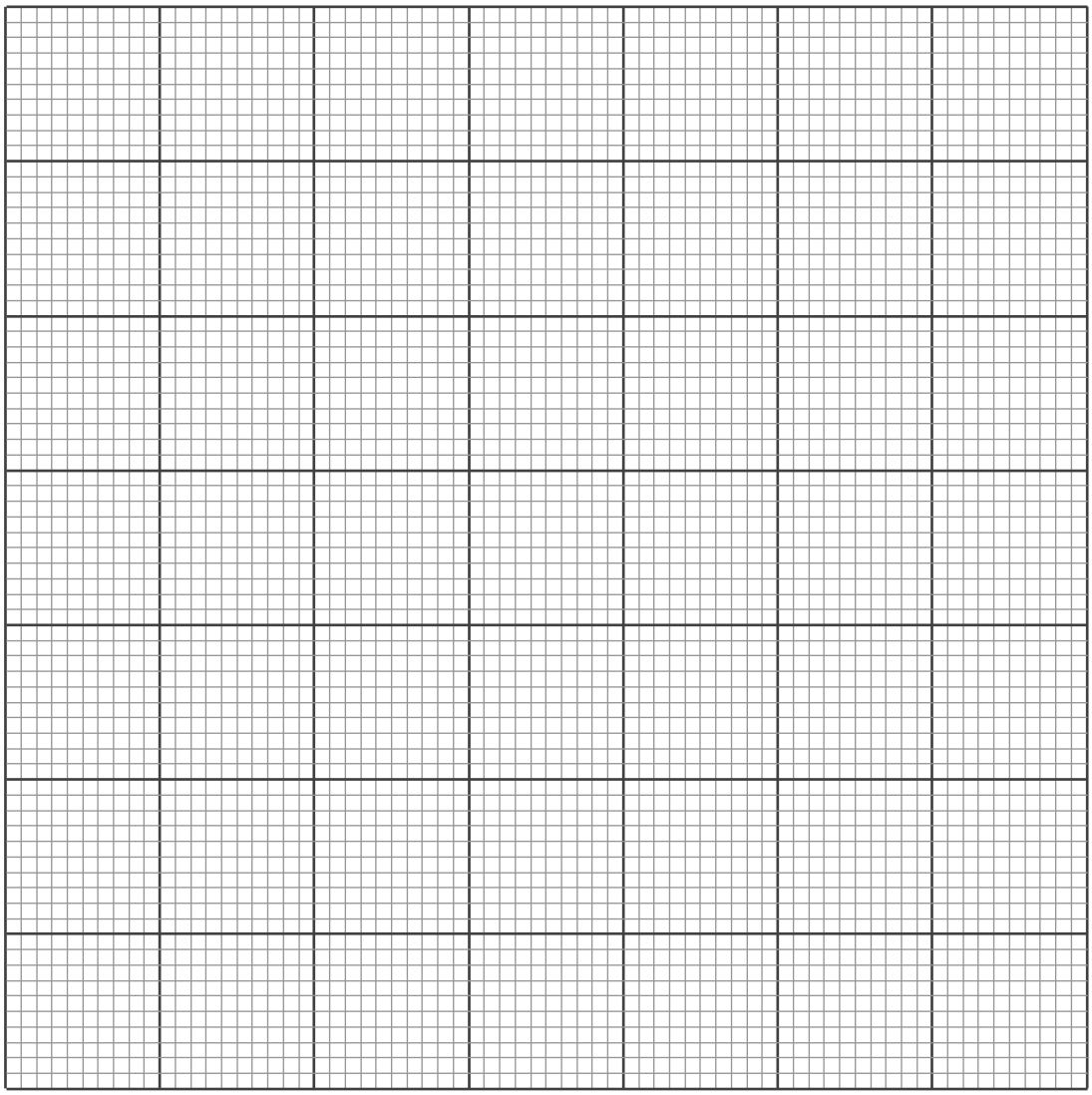
23 In another part of this investigation, pigments were extracted from two more sets of young plants, **A** and **B**. The pigments were extracted as in **Procedure 2** and placed into a colourimeter to measure the absorbance of light using filters to find the absorbance at various wavelengths of light. The higher the absorbance, the more light is absorbed by the pigments.

The results are shown in Table 1.1.

Table 1.1

wavelength of light / nm	absorbance of young plants **A**	absorbance of young plants **B**
400	0.45	0.07
440	0.68	0.21
500	0.38	0.11
540	0.09	0.01
620	0.15	0.01
680	0.45	0.08
740	0.02	0.01

(e) Plot a graph of the absorbance for the young plants against wavelength of light.



[3]

(f) From the graph plotted, suggest what conclusions can be made about the number of pigments and about the concentration of pigments in the two sets of young plants.

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..... [2]

(g) The two sets of young plants **A** and **B** were grown in different conditions.

(i) Based on all your observations and the experimental data available, suggest how the growing conditions were varied.

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(ii) Explain how the results from all your observations and the experimental data support this conclusion.

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.... [2]

Part B

In another investigation, a student was provided with two plant proteases that hydrolyse the amino acid chains of protein in different ways, producing mixtures of single amino acids and peptides of varying lengths:

- An **endoprotease** (**EN**) that hydrolyses peptide bonds between **specific** amino acids within the protein molecule. The enzyme only functions if there is a minimum of two amino acids on each side of the hydrolysis site.
- An **exoprotease** (**EX**) that hydrolyses protein molecules by removing amino acids one at a time from the terminal carboxyl (–COOH) end. This enzyme only functions if the substrate molecule has a minimum of three amino acids.

The student used these enzymes to hydrolyse a protein (**PT**) formed by the linking of two polypeptides.

Fig. 1.2 shows the hydrolysis sites of these two enzymes on this protein.

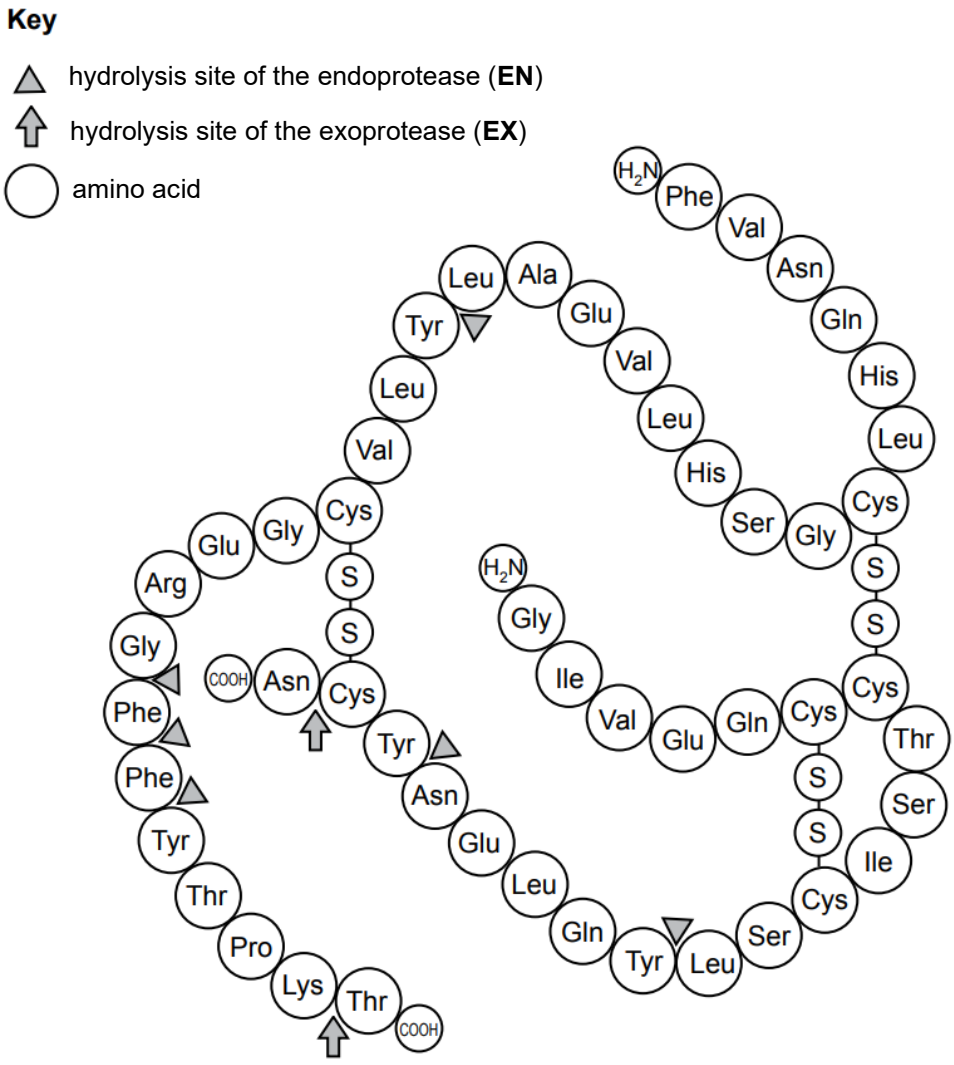

Fig. 1.2

The student carried out some preliminary experiments to find the optimum conditions for complete hydrolysis of **PT**. Key steps include:

- incubating **PT** separately with each of the enzymes
- removing a sample of each mixture, using a micropipette tip, at intervals of 5 minutes
- separating the products of hydrolysis by chromatography
- locating the products of hydrolysis by spraying the chromatograms with a specific dye that stains proteins, peptides and amino acids
- determining the time taken for the **complete** hydrolysis of **PT** by each enzyme.

(h) Design an experiment to compare the **rates** of complete hydrolysis of **PT** by **EN** and **EX**.

Assume that both enzymes would completely hydrolyse **PT** within 60 minutes.

Details of how the chromatograms are prepared need not be included. These have already been described in **Part A**.

In your plan you must use:

- 1% of **EN** solution, prepared in a pH 7.6 buffer
- 1% of **EX** solution, prepared in a pH 7.6 buffer
- an extract of **PT**, prepared in a pH 7.6 buffer
- thermostatically controlled water bath
- pieces of chromatography paper and appropriate solvent
- a spray bottle containing a specific dye that stains proteins, peptides and amino acids.

You may select from the following apparatus and plan to use appropriate additional apparatus:

- normal laboratory glassware, e.g. test-tubes, boiling tubes, beakers, measuring cylinders, graduated pipettes, glass rods, etc.
- syringes
- timer, e.g. stopwatch
- micropipette tips.

Your plan should:

- have a clear and helpful structure such that the method you use is able to be repeated by anyone reading it
- identify the independent variable and the dependent variable
- indicate how the results will be recorded and analysed
- describe the scientific reasoning used to decide the method so that the results are as accurate and repeatable as possible
- use the correct technical and scientific terms.

[9]

Fig. 1.3 shows the chromatograms produced when complete hydrolysis of **PT** by each enzyme had occurred. Fig. 1.3 also shows the time taken for complete hydrolysis.

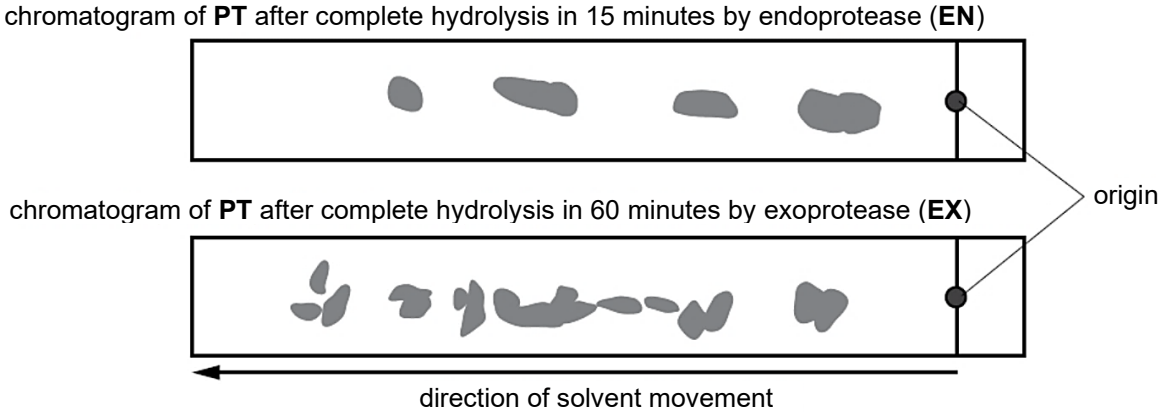

Fig. 1.3

The student concluded that:

1 **EN** worked faster than **EX** because fewer bonds needed to be hydrolysed
2 the products of hydrolysis by **EX** were all single amino acids giving more spots on the chromatogram
3 hydrolysing **PT** with a mixture of **EN** and **EX** would give the same results as for **EX** alone but more quickly.

(i) State **and** explain whether each of these conclusions is supported **or** not supported by all of the information provided about these two enzymes, including the evidence in Fig. 1.2.

conclusion 1: .....

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conclusion 2: .....

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conclusion 3: .....

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..... [3]

[Total: 30]

QUESTION 2

During this question you will require access to a microscope, leaf **L**, stage micrometer and slide **N1**.

(a) You will investigate the stomatal density on the lower surface of a leaf from a plant of *Epipremnum aureum* grown under high light intensity.

You are provided **with a leaf, labelled L.**

Proceed as follows:

1 Cover a small area (about 1 cm^2) of the lower epidermis of **L** with a thin layer of clear nail varnish. Make sure that you do not cover an area with large veins. Repeat this three more times on different areas of the lower epidermis to ensure that you have spare material if necessary.

2 Put **L** to one side for at least 10 minutes to allow the nail varnish to dry.

3 Work through parts **(b)** and **(c)** while you wait for the nail varnish to dry, or go on to other parts of the Question Paper, as appropriate.

4 After allowing the nail varnish to dry for **at least** 10 minutes, carefully use the blade of a fine scalpel to lift one edge of the layer of nail varnish. You may then use forceps to gently peel the layer of nail varnish from the leaf. The layer will still be useful even if it does not come off in one piece. Transfer the layer to a microscope slide. Gently place a cover slip on the thin layer. No water is required.

Fig. 2.1 shows the microscope image of one leaf impression prepared in this way.

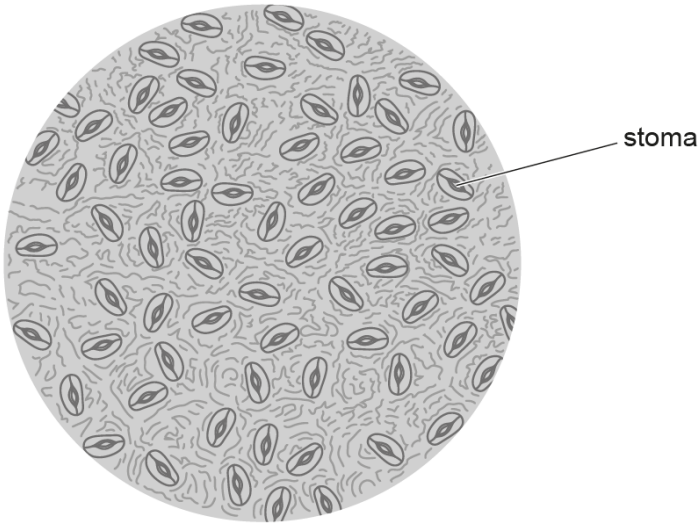

Fig 2.1

(i) Examine the slide using a microscope and locate the stomata. Observe the thin layer using both the low-power and high-power objective lenses, and choose the lens that is most suitable for counting the number of stomata in the field of view.

State which objective lens you have decided to use and give a reason for your choice.

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..... [1]

(ii) Using the objective lens selected in **(a)(i)**, calculate the **mean** number of stomata per field of view for **L**. Show your working.

You should count every stomata for which the **centre** of the stomatal pores is visible in the field of view.

[2]

(iii) Focus the objective lens selected in **(a)(i)** on the stage micrometer provided. The stage micrometer is 10 mm long and has 100 divisions.

Calculate the area of the field of view and record this area in mm^2. Show your working.

area of circle = πr^2

[3]

(iv) Using your results from **(a)(ii)** and **(a)(iii)**, calculate the mean stomatal density per mm^2 for **L**.

[1]

(b) A group of students found a published investigation on the effect of light intensity on stomatal density in the species *Lycopersicon esculentum*.

Two plants of *Lycopersicon esculentum* were selected. One was grown in high light intensity and the other was grown in low light intensity.

The results are shown in Table 2.1.

Table 2.1

leaf number	high light intensity			low light intensity		
	number of stomata x 10^3		leaf area / 10^2 mm^2	number of stomata x 10^3		leaf area / 10^2 mm^2
	upper surface	lower surface		upper surface	lower surface	
1	1634	3131	496	18	1277	160
2	1482	5072	509	10	906	115
3	1865	6365	637	14	1398	171
mean	1660	4856	547	14	1194	149

(i) Deduce a relationship between light intensity and stomatal density based on the results shown in Table 2.1.

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..... [1]

(ii) Using an appropriate pair of stomatal densities, justify your answer in **(b)(i)**.

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(iii) In another investigation carried out by the scientists, the same two plants of *Lycopersicon esculentum*, where one was grown in high light intensity and the other was grown in low light intensity were used.

30 leaves were selected from each plant, and the scientists only investigated the lower surface of the leaves.

The results are shown in Table 2.2.

Table 2.2

	high light intensity	low light intensity
mean stomatal density / mm^{-2}	86 ± 4	80 ± 3

The scientists carried out *t*-test to determine whether there was a significant difference between these means.

The formula for *t*-test is

$$t = \frac{|\bar{x}_1 - \bar{x}_2|}{\sqrt{(\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2})}}$$

Calculate the value of *t* for the mean stomatal density.

Show your working.

[2]

(iv) Table 2.3 shows the critical values at $p < 0.05$ for the *t*-test.

Table 2.3

degrees of freedom	18	20	22	24	26	28	30	> 30
critical value	2.10	2.09	2.07	2.06	2.06	2.05	2.04	1.96

Using your result from **(b)(iii)** and Table 2.3, comment on what the scientists' results in Table 2.2 show and suggest an explanation for any pattern observed.

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..... [4]

(c) Stomata in plants control the movement of gases into and out of a leaf, making carbon dioxide available for photosynthesis. On the other hand, roots in plants ensure there is sufficient uptake of water essential for photosynthesis.

N1 is a slide of a stained transverse section through a plant root.

You are not expected to be familiar with this specimen.

(i) Use the microscope to observe the different tissues in the root on **N1**.

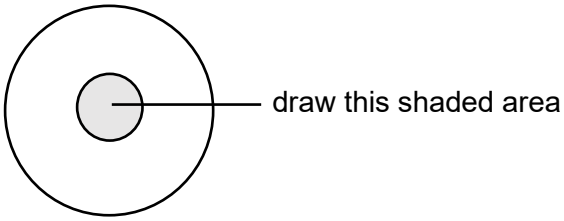

Fig. 2.2

Draw a large plan diagram of the shaded area of the root on **N1** shown in Fig. 2.2. This shaded area contains the vascular system.

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

No cells shown be drawn.

[4]

(ii) Observe the cells in the endodermis (outermost layer of the shaded area) of the root on **N1** shown in Fig. 2.3.

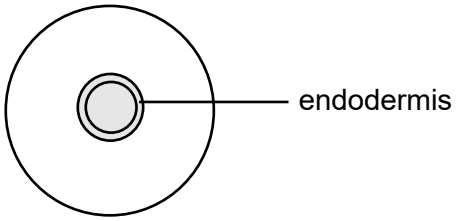

Fig. 2.3

Select **one** group of two adjacent endodermal cells and two other adjacent cells just outside the shaded area.

Each cell must touch at least two of the other cells.

Make a large drawing of this group of **four** cells.

[3]

(iii) Fig. 2.4 is a photomicrograph of a stained transverse section through a different root.

Observe the photomicrograph in Fig. 2.4 and the section on **N1** to identify differences between them.

Complete Fig. 2.4 by:

- identifying **and** annotating **two** differences between the photomicrograph in Fig. 2.4 and the section on **N1**
- using a label line to identify the feature that is different.

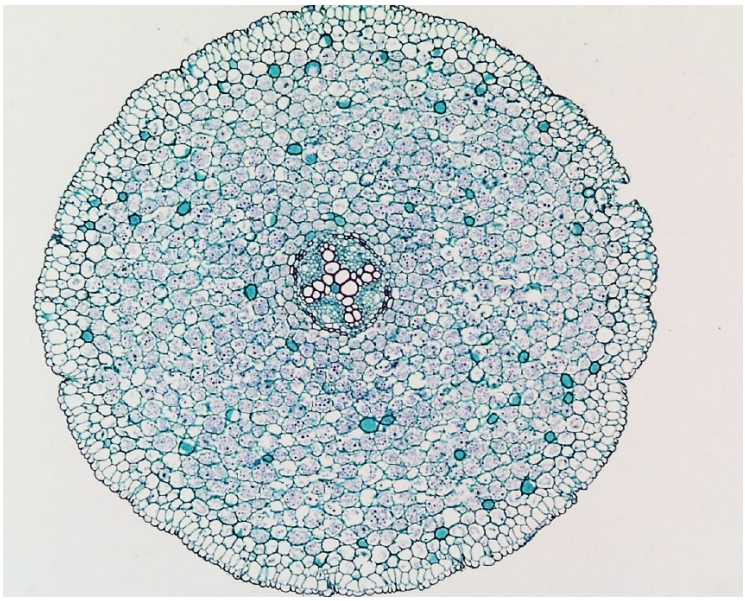

Fig 2.4……..	Fig 2.4 ……..
……	……
N1 …….	**N1** …….
……	……

Fig. 2.4

[2]

[Total: 25]

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