PRACTICAL 1: BOTANY 實作 1: 植物

Student name:	Student code:	Country:

28th International Biology Olympiad

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University of Warwick
United Kingdom

Practical Exam 1

BOTANY

The exam will start and end with a whistle.

You have until the exam begins to familiarise yourself with the microscope. You may use one of the slides on your bench.

Total points: 54
Duration: 120 minutes

GENERAL INSTRUCTIONS

GENERAL INSTRUCTIONS 一般說明

In this practical test you have **TWO hours** to do **FOUR Questions**.

此實作有兩小時，來回答四個問題

You can perform the tasks in any order but note that task 6a** includes a 1 hour incubation step:
除了 **Task 6a (需要有1小時的培養過程)**，各部分沒有操作順序。

Question 1: Plant taxonomy 植物分類

Task 1 – Identify plant species samples and the ploidy of their tissue. (4 Marks)
Task 1 – 鑑定植物樣本的種類和其組織的倍體數 (4分)

Task 2 – Identify flower families and dissect flowers to identify carpel structure. (7.5 Marks)
Task 2 – 鑑定花的科別和解剖花以鑑定心皮的構造 (7.5分)

Task 3 – Prepare stem sections and identify vascular structure and stem organisation. (9 Marks)
Task 3 – 製作莖的切片，並鑑定維管束構造和莖的結構組成. (9 分)

Task 4 – Determine the phylogeny of species used in tasks 1-3. (3 Marks)
Task 4 – 判斷 tasks 1-3 中的物種之親源關係 (3 分)

Question 2: Floral morphology 花的形態

Task 5 – Dissect flowers to determine floral part identity and flower organisation. (10 Marks)
Task 5 – 解剖花來判斷花的各部位和組成結構 (10 分)

Question 3: Seed and embryo development 種子及胚的發育

Task 6 – Dissect siliques to determine the stage of seed development and compare seed development in two plants. (10 Marks).
**Note this has a 1 hour incubation step
Task 6 – 解剖長角果來判斷其中的種子發育階段，並比較兩種植物的種子發育。 (10 分) 注意這部分需要有1小時的培養過程

Question 4: Root morphology 根的形態

Task 7 – Observe and determine types of root hair and root development in seedlings. (8 Marks)
Task 7 – 觀察並判斷小苗中根毛和根的發育之類型 (8 分)

Task 8 – Determine the manner of inheritance of root development genes. (2.5 Marks)
Task 8 – 從根的發育表徵來判斷基因型 (2.5 分)

This is a test of sample handling and manipulation, fine dissection skills, observation and reasoning.
此實作測試樣本操作及細微解剖技術，觀察及解釋等能力。

Good luck!

Important Information: 重要訊息

- Please remember to write your name, your student code and your country in the given boxes.
  請記得寫姓名、編號及國家
- Write your answers in this question booklet. Only the answers given on this question booklet will be evaluated.
  把答案寫在試題本上。只有在試題本上的答案才會被批改。
- Make sure that you have received all the materials and equipment listed. If any of these items are missing, please raise your flag immediately.
  確定你有所有材料及器材。若有缺少，請立刻舉旗!
- During experiments, ensure that you handle equipment and samples properly. Any spilled solutions, samples or equipment damaged by you will not be replenished.
  實作期間，請小心正確操作所有器材及樣品。若有溶液因傾倒變少，或用壞的器材，將不再補充或更換。
- Stop answering and put down your pen immediately when the whistle sounds at the end of the exam.
  實驗結束、鈴聲響起，請立即停止作答並放下筆。
- Leave the question booklet on your desk at the end of the exam.
  把所有試題本放在信封中
- No paper, materials or equipment should be taken out of the laboratory.
  不可將紙張、材料或器材帶出考場

MATERIAL AND EQUIPMENT 材料及儀器

Material and equipment 材料及儀器

Make sure that you have received all the materials and equipment listed for each task. If any of these items are missing, please raise your flag.
檢查確定所有材料及儀器，若有缺少，請舉旗通知監考人員

Required for all questions 適用於所有問題

- Stereo (Dissection) Microscope 解剖顯微鏡
- Compound microscope 複式顯微鏡
- Hand Lens 放大鏡
- Straight teasing needles 探針
- Curved fine point forceps 彎頭細尖鑷子
- Fine point forceps 細尖鑷子
- Small scissors 小剪刀
- Waste beakers 廢物瓶
- Permanent marker pens 簽字筆
- Roll of white tissue 捲筒衛生紙
- Powder free disposable nitrile gloves 手套
- Lab Coat 實驗衣
- Safety Goggles 護目鏡
- 2 blank paper sheets for notes 2張空白計算紙
- Flag to call the lab assistant 需助教協助用的旗子
- **An English translation of this paper is available upon request.** 若需英文試卷，可向監試人員提出需求

Question 1: Plant taxonomy 植物分類

Plant material 植物材料

- 7 plant specimens labelled A, B, C, D, E, I, and J
  標示 A, B, C, D, E, I, and J 的七種植物
- 5 flowers labelled E, F, G, H,and I (specimens from the same species (E and I) are used in tasks 2 AND 3)
  標示 E, F, G, H,and I 的五種植物的花 (其中 E and I 材料的物種還會在 Tasks 2 AND 3 用到)
- 3 microscope slides of plant sections labelled K, L, and M
  三片標示為 K, L, and M 的植物永久玻片

Solutions and reagents 溶液和藥品

- 10ml Solution A (Toluidine blue stain) A溶液是藍色染劑
- 10ml Solution B (Distilled water) B溶液是蒸餾水

Technical material 本單元相關之操作用具

- Plastic teat pipettes 塑膠吸管
- Safety razor blades 單面刀片
- 12 microscope slides and 12 cover slips (total for all tasks)
  12個載玻片及12個蓋玻片 (所有 tasks 總共需要量)
- Disposable jars for used slides/razor blades
  用來裝用過的玻片和刀片的廢棄瓶
- 2 petri dishes 兩個培養皿
- Timer 計時器

Question 2: Floral morphology 花的形態

Plant material 植物材料

- 3 tubes containing inflorescence (flowering stem) tips with flowers from 3 different *Arabidopsis thaliana* genetic lines. Labelled O, P, and Q
  三管分別裝有來自三種不同阿拉伯芥基因型的花序，分別標示為 O, P, and Q

Question 3: Seed and embryo development
Question 3: 種子和胚的發育

Plant material 植物材料

- 2 tubes containing inflorescences (flowering stems) from 2 different *Arabidopsis thaliana* genetic lines that are developing seeds. Labelled R, and S.
  兩管分別裝有來自兩種不同阿拉伯芥基因型的花序，其包含發育中的種子，分別標示為 R, and S

Solutions 溶液

- 1.5 ml Solution C (Hoyer's solution (please note this is an irritant chemical solution))
  溶液 C 是 Hoyer's solution **(注意: 此化學溶液有刺激性)**

Technical material 本單元相關之操作用具

- Electrical tape (yellow) 黃色絕緣膠帶
- Hypodermic needles for dissection 皮下注射針頭用來解剖操作
- Safety razor blades 單面刀片
- 12 microscope slides and 12 cover slips (total for all tasks)
  12個載玻片及12個蓋玻片 (所有 tasks 總共需要量)
- Disposable jars for used slides/razor blades
  裝用過的玻片及刀片的廢棄瓶
- Double sided sticky tape (white) 雙面膠帶 (白色)
- Timer 計時器
- 15 cm ruler 量尺

Question 4: Root morphology 根的形態

Plant material 植物材料

- 5 agar plates of seedlings: 5 different *Arabidopsis thaliana* genetic lines labelled T, U, V, W, and X
  五個裝有小苗的培養皿: 五種不同的阿拉伯芥基因型，標示為T, U, V, W, and X

Technical material本單元相關之操作用具

- 12 microscope slides and 12 cover slips (total for all tasks)
  12個載玻片及12個蓋玻片 (所有 tasks 總共需要量)

QUESTION 1: PLANT TAXONOMY 植物分類

(23.5 marks) 共 **23.5**分

Introduction 簡介

As plants have evolved they have acquired morphological characteristics related to the function that they provide. Species can be identified by their characteristics.
植物依其所具有之形態特徵對應其功能而演化。故可用其特徵來鑑定物種。

Task 1

Species ploidy identification 物種倍體數的鑑定

You are provided with 4 plant specimens labelled **A, B, C & D**. Examine them carefully using the dissecting microscope. Based on your observations complete the Specimen table below using ONE of the options below for each sample. You can use each code more than once in the table.
有四種標示為 **A, B, C & D** 的植物樣本，用解剖顯微鏡仔細檢查。根據你的觀察，在下面的樣本表格中，每個填入一個選項，每個編號可能會重複使用。

Specimen table key 樣本表格的答案參考編號

Species type 物種類別	**Species Code** 物種編號
Angiosperm 被子植物	1
Moss 苔類	2
Conifer 松柏植物	3
Fern 蕨類	4

Ploidy level 倍體數	**Ploidy Code** 倍體數編號
Haploid: if the tissues or cells in the specimen are from all or part of one type of gametophyte only. 單倍體: 若樣本的組織或細胞是全部來自一種配子體，或屬於配子體的一部分。	n
Diploid: if the tissues or cells in the specimen are from all or part of one sporophyte generation only. 二倍體: 若樣本的組織或細胞是全部來自一個孢子體世代，或屬於一個孢子體世代的一部分。	2n
Tissues or cells from all or part of two sporophyte generations. 組織或細胞是全部來自兩個孢子體世代，或屬於兩個孢子體世代的一部分。	2n+2n
Tissues or cells from all or part of one sporophyte generation and two types of gametophytes. 組織或細胞是全部來自一個孢子體世代，或屬於一個孢子體世代的一部分，還有包括兩種配子體。	2n+n+n
Tissues or cells in the specimen are from all or part of one sporophyte generation and one type of gametophyte. 組織或細胞是全部來自一個孢子體世代，或屬於一個孢子體世代的一部分，還有包括一種配子體。	2n+n

*Specimen table **(4 marks)** 樣本表格 **(4 分)***

Specimen 樣本	**Species code** 物種編號	**Ploidy level code** 倍體數編號
A		
B		
C		
D		

Task 2

Floral structure 花的構造

You are provided with 5 flowering specimens labelled **E, F, G, H, & I**. Examine them carefully using dissection and the dissecting microscope (only use up to 20x magnification). Based on your observations complete the flower specimen table below using ONE of the options within each column for each sample. You can use each code more than once in the table.

有五種標示為 **E, F, G, H, & I** 的花樣本，用解剖顯微鏡仔細檢視 (最高只放大至20x)。根據你的觀察，在下面的樣本表格中，每個填入一個選項，每個**編號可能會重複使用**。

Flower specimen key 花樣本的參考答案

Family 科	**Family code** 科的編號
Asteraceae 菊科	1
Brassicaceae 十字花科	2
Lamiaceae 唇型科	3
Fabaceae 豆科	4
Onagraceae (not in syllabus) 柳葉菜科 (未列入"須認識科名"中)	5
Magnoliaceae 木蘭科	6

Gynoecium position 雌蕊位置	**Gynoecium code** 雌蕊編號
Hypogynous flower (superior ovary) 下位花 (子房上位)	7
Epigynous flower (inferior ovary) 上位花 (子房下位)	8
Perigynous flower (neither superior or inferior ovary) 周位花 (不是子房上位或下位)	9

Carpel structure 心皮構造	**Carpel code** 心皮編號
Single ovary of one carpel only 單一心皮所構成的單一子房	10
Ovary of fused carpels 多個心皮癒合而成的子房	11
Many separate carpels (compound flower) 多個分離的心皮 (複合型的花)	12

*Flower specimen table **(7.5 marks)** 花樣本表格 **(7.5 分)***

Specimen 樣本	**Family code** 科編號	**Gynoecium position code** 雌蕊位置編號	**Carpel structure code** 心皮構造編號
E			
F			
G			
H			
I			

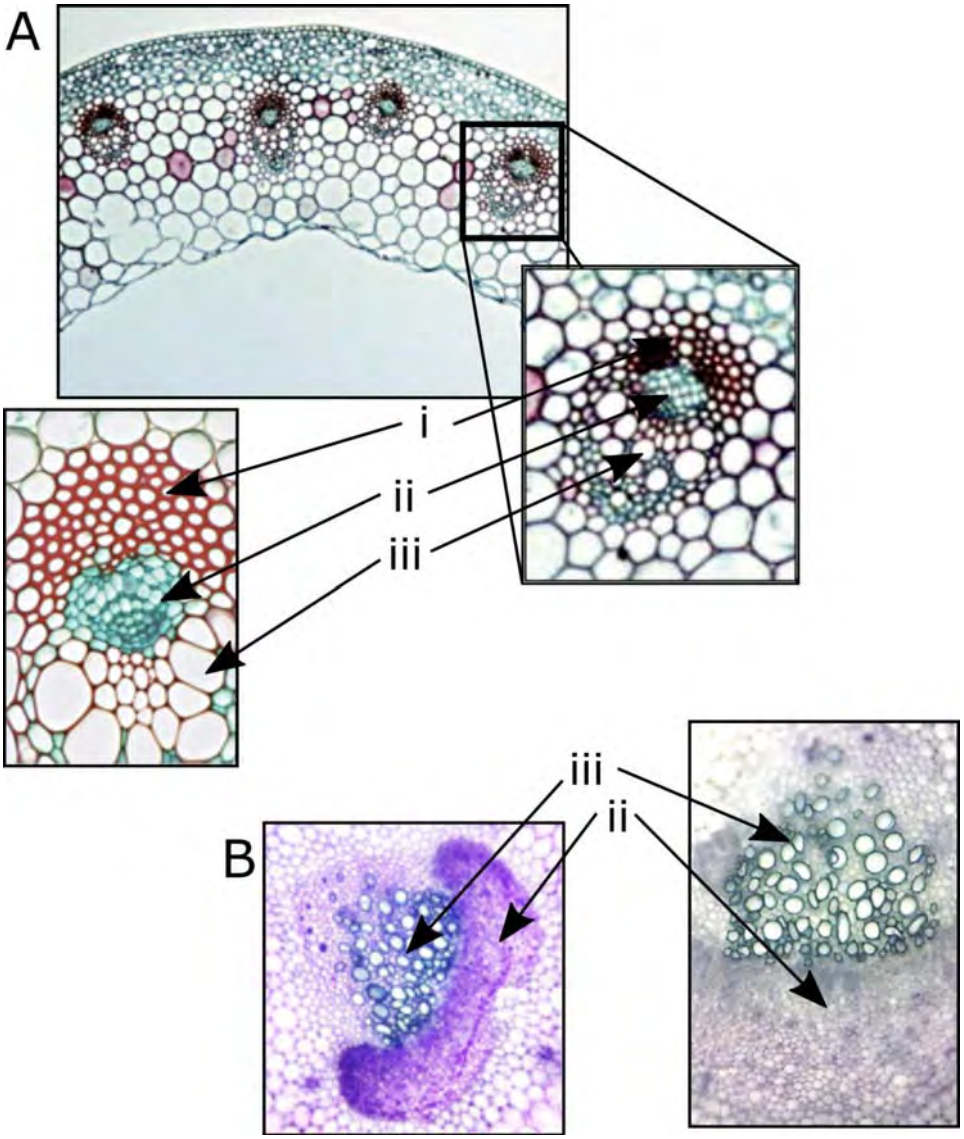
Task 3

Introduction 簡介

Higher plants stems are comprised of plant vascular tissues made up of xylem vessels, phloem sieve tube elements and companion cells, and schlerenchyma fibres. Xylem vessels, phloem cells and sclerenchyma fibres can be identified by their structure.
較高等植物的莖之維管束中，由木質部導管、韌皮部篩管及伴細胞、以及厚壁纖維所組成。這些組織細胞可由其構造特徵來判辨。

Feature 特徵	**Xylem vessels** 木質部導管	**Phloem** 韌皮部	**Sclerenchyma fibres** 厚壁纖維
Function 功能	Transport of water and mineral ions Structural support 輸送水及礦物鹽、結構性支持	Transport of dissolved carbohydrates 輸送溶解的碳水化合物	Structural support 結構性支持
Structure 構造	Hollow tubes made of many connected cells 多個細胞相接的中空管子	Hollow tubes made of many connected cells 多個細胞相接的中空管子	Separate cells joined together to form fibres 多個細胞聚集成較粗的纖維束
Dead/alive 死/活	Dead cells 死細胞	Sieve tube elements kept alive by connections to companion cells 與伴細胞相連的篩管細胞可維持是活的狀態	Dead cells 死細胞
Cell wall 細胞壁	Thickened and possess lignin 加厚且具木質素	No special modifications (plasmodesmata between sieve tube element and companion cells, also sieve plates at the end of cells) 沒有特別的改變 (篩管細胞及伴細胞之間有原生質絲，細胞端壁為篩板)	Thickened and possess lignin 加厚且具木質素
Cell contents 細胞內含物	Completely hollow – some perforated or slitted end plates of cells remain 完全中空 - 有些穿孔板是完全打通或留有細縫	Hollow centre with thin layer of cytoplasm around the edge. Sieve plates at the end of each cell. 中央是空心的，邊緣仍有薄的原生質，細胞端壁有篩板	Hollow dead cells 中空的死細胞

You can see examples of these cells and the way the vascular tissue is organised in these pictures of transverse sections of buttercup (A) and celery (B).
例如:下方的照片圖分別為毛艮 (A) 及芹菜(B) 的橫切，可見維管束組織細胞的結構組成。



Transverse sections of stems. i = sclerenchyma fibres, ii = phloem, iii = xylem. A = buttercup, B = celery.
莖的橫切。i = 厚壁纖維，ii = 韌皮部， iii = 木質部。A =毛茛 ，B = 芹菜

Vascular tissues of the stem are organized in continuous strands in several ways, depending on the spatial location of the cells:
根據細胞的空間位置，莖的維管束組織可構成多種連續性束狀結構。

- In **bicollateral** vascular bundle organization the phloem is located towards both the inside and the outside and the xylem is located between the phloem poles.
  在複並列維管束的結構中，韌皮部位在內側及外側，而木質部夾在兩韌皮部之間
- In **collateral** vascular bundle organization the phloem is located towards the outside and the xylem is located towards the inside.
  在並列維管束的結構中，韌皮部位在外側，而木質部在內側
- In **concentric amphivasal** vascular bundle organization the xylem encloses the phloem.
  在同心環木維管束的結構中，木質部包圍韌皮部
- In **concentric amphicribral** vascular bundle organization the phloem surrounds the xylem.
  在同心環韌維管束的結構中，韌皮部包圍木質部
- In **monocotyledons (monocots)**, there are **scattered** vascular bundles, dispered over the stem, although more towards the periphery
  單子葉植物中，維管束散生，但近周圍的維管束較多
- In **dicotyledons (dicots)**, there are **reticulate** vascular bundles, arranged in the stem to form a cylinder
  雙子葉植物中，在莖中的維管束構成環狀網柱
- In **woody** species, thickening of the stem occurs, visible as **annual ring** formation.
  在木本物種中，莖橫向加粗，可見年輪

Stem rigidity is maintained either by turgor pressure inside cells, or secondary wall material (termed secondary thickening).
莖的堅硬程度是由細胞內的膨壓或次生細胞壁 (稱為次生壁加厚) 來決定

You are provided with 3 pre-prepared stained transverse stem sections labelled K, L, & M. You are also provided with 3 plant specimens labelled E, I & J (Specimens from the same species (E and I) are used in tasks 2 AND 3). Prepare and stain transverse sections of stems from these plants.
三片永久玻片 (分別標示為 K, L, & M) 是染色後之莖橫切。另有三種植物樣本 (分別標示為 E, I & J)，其中樣本 E and I 和在 Tasks 2 AND 3 的材料為同種植物。製作這三種植物莖的徒手橫切片並染色。

1. Hold the stem of the plant in a vertical angle between the thumb and the index of your non-dominant hand.
   用拇指及食指以垂直方向握住植物莖
2. Hold the razor with your dominant hand and start cutting transverse sections of the stem, as thin as possible.
   以慣用的手來切莖，切出薄的橫切片
3. In one petri dish, add a few drops of solution A (blue stain).
   在一個培養皿中加幾滴藍色染劑 (solution A)
4. Place the transverse sections into the stain and stain for 3-5 minutes; staining time depends on the thickness of the section.
   將橫切下來的切片放入染劑中，染 3-5 分鐘；依切片厚度來調整染色時間
5. In a second petri dish, add Solution B (distilled water). Once the staining is completed, transfer the sections into the Solution B, swirl and remove the Solution B. Repeat until excess stain washes out.
   在第二個培養皿中，加溶液 B (蒸餾水)，將染色完全的切片移入水中清洗，換水，直至洗盡多餘染劑
6. Carefully transfer the cross-section to a microscope slide, mount in water, add a coverslip over the section and label the slide with the relevant letter (E, I & J).
   小心將染好的橫切片置於載玻片上，加水製成水埋玻片。並用簽字筆標示玻片 (E, I & J)

Examine the transverse sections on both the pre-prepared slides (K,L,M), and those that you have prepared, under low and medium magnification, using both the dissecting and compound microscopes. Based on your observations complete the following table using the ONE code that you think best fits the sample character. You can use each code more than once within the table.
檢視永久切片 (K,L,M)及你製作的水埋玻片，用解剖及複式顯微鏡在低倍及中倍放大倍率之下來觀察。根據你觀察，將最符合樣本特徵之一個編號填入表格中，編號可重複使用。

Section specimen key 切片標本的參考答案

Vascular bundle type 維管束類型	**Type code** 類型編號
Bicollateral 複並列	1
Collateral 並列	2
Concentric amphivasal 同心環木	3
Concentric amphicribral 同心環韌	4

Bundle organisation 維管束結構	**Bundle code** 維管束編號
Arranged in a ring 排列成環狀	5
Scattered 散生	6
Annual rings 年輪	7

Stem rigidity 莖的堅硬程度	**Rigidity code** 堅硬程度編號
Turgor pressure 膨壓	8
Secondary thickening 次生壁加厚	9

Section table ***(9 marks)*** *切片表格* ***(9 分)***

Specimen 樣本	**Vascular bundle type code** 維管束類型編號	**Vascular bundle organisation code** 維管束結構編號	**Provision of stem rigidity code** 堅硬程度編號
E			
I			
J			
K			
L			
M			

Task 4

Taxonomy 分類

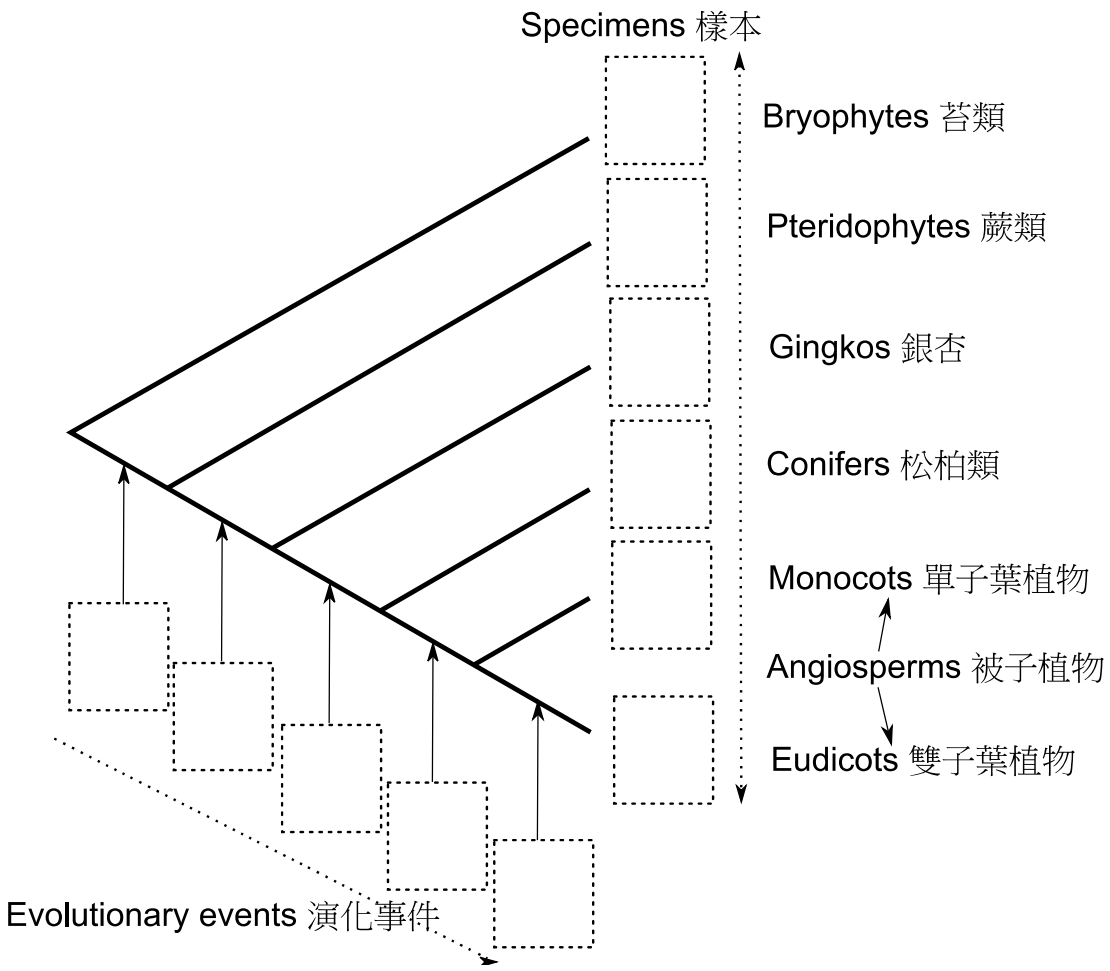
For plant species A-E & I-L, annotate the tree with the most likely position of each specimen species A-E and I-L in the boxes. Each box may include one or more than one specimen, or no specimens.
將植物物種 A-E & I-L 填入樹狀圖中的適當位置。每個空格可包括一或多個樣本，或者沒有對應的樣本。

In addition, annotate the tree with the presence of three evolutionary events (each box may include one or more than one event, or no events):
此外，在樹狀圖上註記三項演化事件 (每個空格可包括一或多個樣本，或者沒有對應的樣本。)

I. Development of first flowers 首度有花的發育

II. Development of first vasculature 首度有維管束組織的發育

III. Development of first seeds 首度有種子的發育



Taxonomic tree ***(3 points)*** *分類樹狀圖* ***(3 分)***

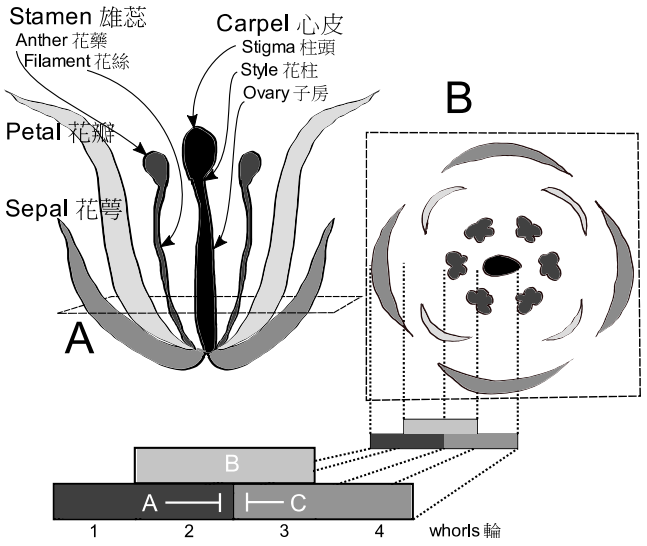
QUESTION 2: FLORAL MORPHOLOGY 花的形態

(10 marks) 10 分

Introduction 簡介

Wild-type eudicotyledon flowers are comprised of four whorls of floral organs in the following order (outside to inside): sepals, petals, stamens, carpels. The figure below shows you the wild-type floral organ structure of *Arabidopsis thaliana* in longitudinal (A) and cross section (B). The floral organ identity of each whorl is determined by the action or interaction of three classes of 'homeotic' gene activity, A, B, C; as shown.

真雙子葉植物花的野生型包括兩輪的花器(floral organ)，其順序由外而內是: 花萼、花瓣、雄蕊、心皮。下圖顯示阿拉伯芥野生型的花器之縱剖面(A) 及橫剖面(B)。每一輪花器是由三群同源區基因 A, B, C 的作用 ('homeotic' gene activity, A, B, C) 本身作用或兩兩交互作用來決定。



- Expression of gene A specifies sepals.
  表現基因 A 會產生花萼
- Expression of genes A and B together specifies petals.
  同時表現基因 A and B 會產生花瓣
- Expression of genes B and C together specifies stamens.
  同時表現基因 B and C 會產生雄蕊
- Expression of gene C specifies carpels.
  表現基因 C 會產生心皮
- Genes A and C repress expression of each other, thus are mutually exclusive.
  基因 A and C 會互相抑制其表現，故屬互相排斥

Task 5a

You have been provided with inflorescence (flowering stem) tips with flowers from three specimens (labelled O, P & Q) from either a wild-type or a knockout mutant *Arabidopsis thaliana* plant. Dissect the flowers and observe the floral organ structures using a range of magnifications. Complete the table below using the codes corresponding to the descriptions below. Choose ONE code for each specimen feature that best describes the phenotype; you can use each code more than once.

三種花序樣本 (O, P & Q)，可能是阿拉伯芥的野生型或剔除突變株。使用不同放大倍率來解剖它們的花並觀察花器構造。在下表中填入適當編號對應以下描述。選擇一個編號對應每個樣本，以最適描述其表現型。每個編號可重複使用。

Organ identity codes 花器辨識的編號

Organs present 存在的花器	**Organs code** 花器編號
Sepals, petals, stamens and carpels 花萼、花瓣、雄蕊及心皮	1
Sepals only 只有花萼	2
Stamens and carpels only 只有雄蕊及心皮	3
Sepals and stamens only 只有花萼及雄蕊	4
Sepals and petals only 只有花萼及花瓣	5
Sepals and carpels only 只有花萼及心皮	6

Classes of gene activity 基因作用分群	**Gene code** 基因編號
A, B, C	7
A, B	8
A, C	9
B, C	10
A	11
B	12
C	13

Genotype 基因型	**Genotype code** 基因型編號
Wild-type 野生型	14
Mutant 突變株	15

*Floral organ identity table **(9 marks)** 花器辨識表 (**9** 分)*

Specimen 樣本	**Organ identity code** 花器辨識編號	**Homeotic gene activity code** 同源區基因作用編號	**Genotype code** 基因型編號
O			
P			
Q			

Task 5b

Complete the table below choosing ONE appropriate code for each answer. You can use each code more than once.
選擇一個適當的編號，填入下表。每個編號可重複使用。

Phenotype codes 基因型編號

Phenotype 基因型	**Phenotype code** 表現型編號
Leaves in all four whorls 四輪皆為葉片	1
Sepals in all four whorls 四輪皆為花萼	2
Petals in all four whorls 四輪皆為花瓣	3
Carpels in all four whorls 四輪皆為心皮	4
Stamens in all four whorls 四輪皆為雄蕊	5

(1 mark) 1 分

	Phenotype code 表現型編號
What is the flower phenotype in a homozygous knockout BC double mutant? 哪個花的表現型屬於同型合子剔除 BC 的雙突變株	
What is the flower phenotype in a homozygous knockout ABC triple mutant? 哪個花的表現型屬於同型合子剔除 ABC 的三突變株	

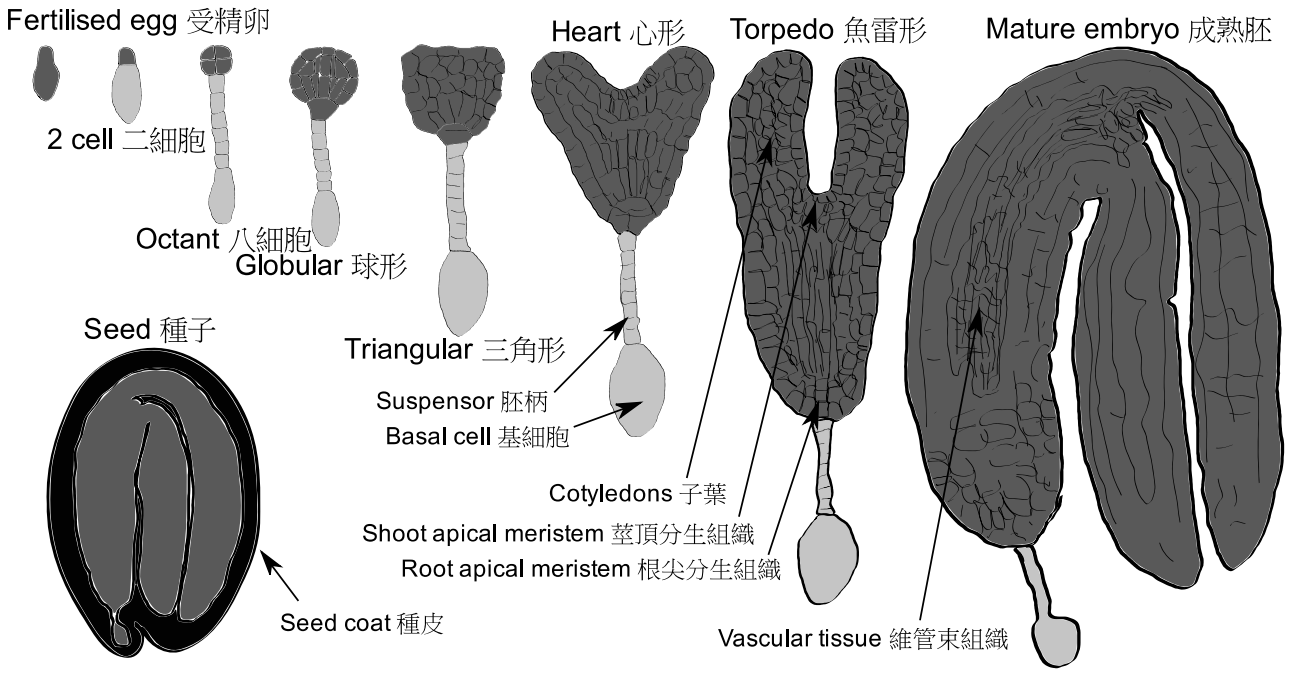
QUESTION 3: SEED AND EMBRYO DEVELOPMENT 種子及胚的發育

(10 marks) 10 分

Introduction 簡介

In wild-type plants, after double fertilisation, each ovule in the carpel of *Arabidopsis thaliana* develops into a seed. As the many seeds develop, the carpel structure enlarges and is termed a silique (seed pod). *Arabidopsis thaliana* plant inflorescence (flowering stem) development is indeterminate, thus on a single plant there are many flowers and siliques at a range of developmental stages (see image of *Arabidopsis thaliana* inflorescence below). Within each developing seed, the embryo progressively grows and develops, and a number of stages have been named based on the morphology (see figure below).

阿拉伯芥野生型植物中，在雙重受精之後，心皮中的每個胚珠發育成種子。當許多種子發育時，心皮變大而形成果實稱為長角果。阿拉伯芥的花序是無限發育，所以一棵植株中會有多朵花，也會有不同發育階段的長角果(如下花序圖所示)。在發育的種子中，胚逐漸生長發育，且有一系列的發育階段，依其形態命名如下圖所示。



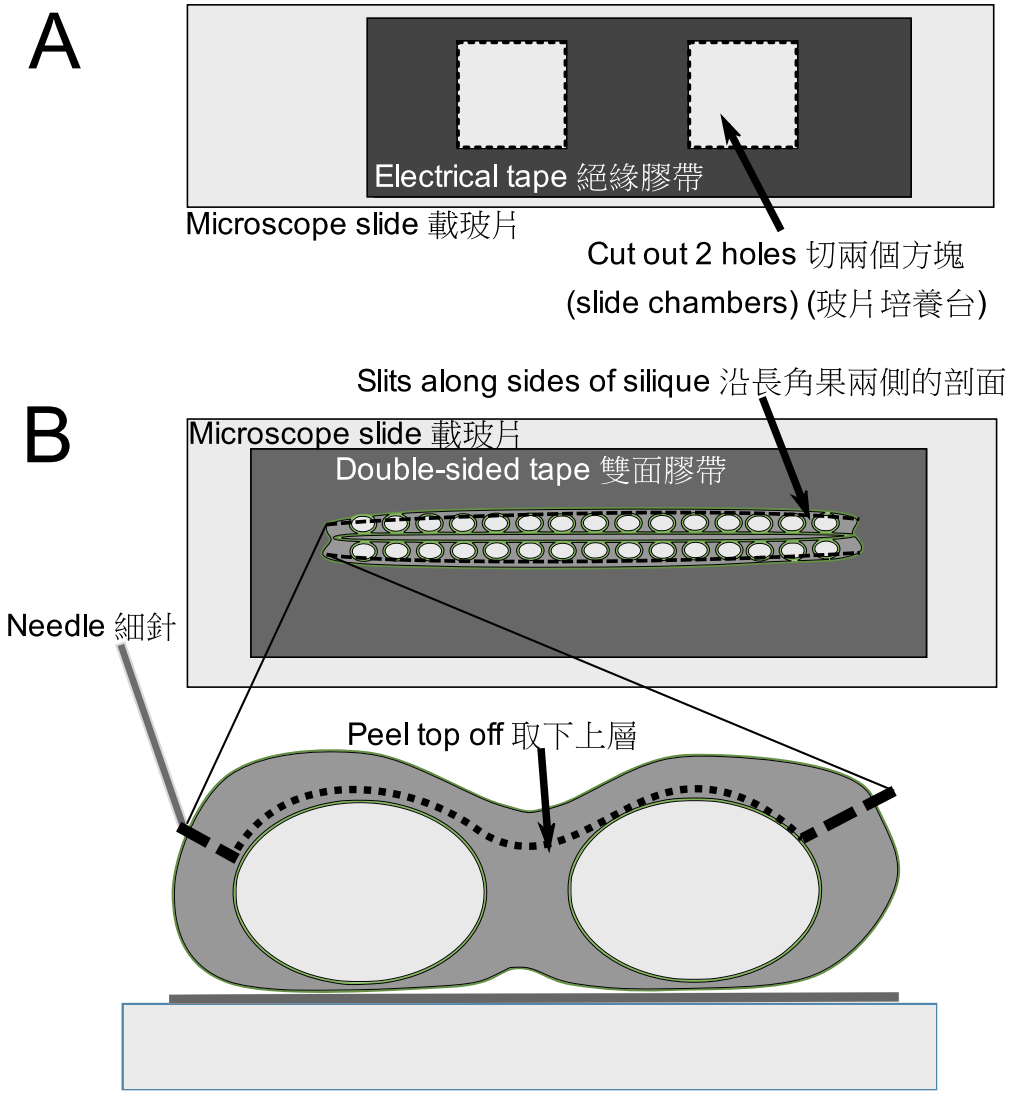
Arabidopsis embryo developemnt 阿拉伯芥的胚發育

Silique age progression. i = youngest flowers, ii = older embryos and seeds.
長角果成熟過程，i = 最年輕的花，ii = 較老的胚及種子

Task 6a
You have been provided with 2 tubes containing inflorescences that are developing siliques of *Arabidopsis thaliana* plants labelled R and S. These are different plant lines. Dissect TWO 1.3-1.7cm long siliques from plant R and TWO 1.3-1.7cm siliques from plant S as described below; the silique is attached by a stalk to the plant stem - only measure the silique length. *You must dissect 1.3-1.7cm long siliques as embryo development at this stage will enable you to observe clear differences between plants R and S.*
R and S兩管中分別有具不同發育階段長角果的花序，這是兩種不同突變株。分別從R and S植株中，挑選兩個1.3-1.7 cm 長 (不包括果柄的長度) 的果實來解剖(操作步驟描述於下)。*你必須解剖 **1.3-1.7 cm** 的長角果，因為在此階段才能明顯區分出 **R and S**兩植物的胚發育。*

Silique dissection procedure 長角果的解剖步驟

1. Make two slide chambers. You will use these in step 4. To do this, first cut a ~4cm strip of the yellow electrical tape and stick it onto a microscope slide. With a razor blade, cut out and remove two small squares from this strip and ensure that the cut edges are flat.
製作兩個玻片培養台(slide chambers)。你會在步驟 4中需要用到。
首先，切一段約 4 公分的黃色絕緣膠帶，把它貼在其中一片載玻片上。然後用刀片從這玻片上割除兩小塊正方形黃膠帶，並確定切下的邊緣平整。(如圖A)
2. Stick a 5cm long piece of double sided sticky tape on to a second microscope slide. Mount the siliques on the sticky tape so that each silique is flat. Observing the slide under a dissecting microscope, use a hypodermic needle to remove the top layer of silique tissue, without damaging the seeds within. Removing this in two strips, on either side of the central silique vein is recommended; the figure below illustrates a cross section of a mounted silique, showing the position of the hypodermic needle for dissection.
取5 cm長的雙面膠帶黏在另一片載玻片上。將兩個長角果黏在雙面膠上，使其扁平面黏住。在解剖顯微鏡下觀察這玻片，並使用皮下注射針頭小心取下長角果上層的果皮，不要傷到裡面的種子。移除上層果皮後可看到種子在中央薄膜的兩側，下圖B指出黏在玻片上長角果橫剖面，顯示如何用細針頭來取下長角果上層的果皮。
3. Observing the slide under a dissecting microscope, record the number of seeds and the number of aborted seeds (if any) within the silique.
在解剖顯微鏡下觀察這玻片，記錄長角果中的種子數以及敗育(若有)種子數
4. Wearing gloves, pipette a drop of Solution C (Hoyer's solution) into each slide chamber. *NB Solution C is an irritant so do not get it on your skin.* Using the hypodermic needle or a teasing needle, transfer all of the seeds from each silique into Solution C in the prepared slide chamber; do this under the dissecting microscope, or by eye. Transfer seeds from plant R into one slide chamber, and seeds from plant S into the other slide chamber.
戴手套，用微量滴管吸取一滴溶液 C (Hoyer's solution) 在每個玻片培養台上，*注意溶液C有刺激性，不要碰到皮膚*。在解剖顯微鏡下或肉眼觀察，用細針頭或探針將每個長角果的所有種子置於玻片培養台上的溶液 C中。兩個玻片培養台分別放植物 R 或 S 的種子。
5. Place a cover slip over the seeds. Store the slides at room temperature for **1 hour** to enable the tissue to be cleared (this reaction makes the tissue more transparent so you can see the developing embryos through the seed coat).
將這些種子蓋上蓋玻片，讓這兩玻片在室溫下培養1小時，讓組織變透明。(此反應可使組織變得較透明，以便透過種皮也可看到發育中的胚)
6. Observe the samples at high magnification using a compound microscope (e.g. you can see a heart-shaped embryo in this cleared seed).
在複式顯微鏡的高倍率放大之下觀察樣本，(例如: 在此透明種子中，你可看到心形胚)



Silique dissection 長角果的解剖

Using an average of both siliques dissected, record the number of developing seeds per silique within each plant line in the table below. Then examine the stage of embryo development for the seeds within the siliques. Record the % of seeds at each stage of embryo development.
記錄下表兩種突變株中，所解剖的兩個長角果之種子數，取其平均。然後檢視果實中的種子胚發育階段，記錄每個階段所佔的百分比。

Silique morphology ***(4 marks)*** *長角果形態* ***(4 分)***

Plant 突變株	**Average number of seeds** 平均種子數	**% globular stage** 球形期	**% heart stage** 心形期	**% torpedo stage** 魚雷形期	**% mature stage** 成熟期
R					
S					

Task 6b
One of the two lines has abnormal (non wild-type) seed development. Define the abnormality and it's frequency. Based on your observation of the number and stage of abnormal embryos, what is the most likely explanation for the abnormal embryos?
兩突變株中，有一個具不正常(非野生型)種子發育，判定此不正常及其頻率。根據所觀察的不正常胚數以及其所在發育階段，此不正常胚應如何解釋?

Complete the table below, choosing ONE code for each question.
完成下表，每個問題填入一適當編號。

Development codes 發育的編號

Seed defect 種子缺失	**Defect code** 缺失編號
Embryo pattern formation is disrupted 胚型的形成遭破壞	1
Seeds lack embryos 種子缺胚	2
Seeds have multiple embryos 種子具多胚	3
Seeds have enlarged embryos 種子具有變大的胚	4

Approximate frequencies of abnormal embryos 不正常胚的約略頻率	**Approximate frequency code** 約略頻率編號
0%-33%	5
34%-66%	6
67%-100%	7

Cause of embryo abnormality 不正常胚的起因	**Cause code** 起因編號
Double fertilisation 雙重受精	8
Development of a non-embryonic cell 非胚細胞的發育	9
Spontaneous 自然發生	10

(6 marks) 6 分

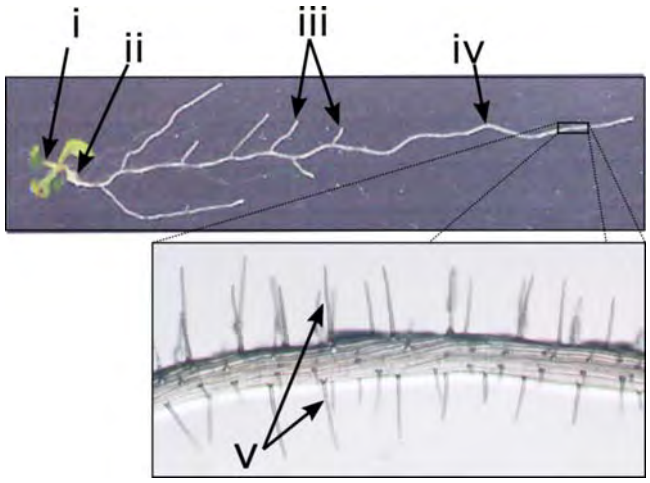
	Which plant line has abnormal development? (R or S) 哪個植物株具不正常發育? (R or S)	Defect code 缺失編號	Approximate frequency code 約略頻率編號	Cause code 起因編號
Answer				

QUESTION 4: ROOT MORPHOLOGY 根的形態

(10.5 marks) 10.5 分

Introduction 簡介

Plant roots are composed of a primary root and a number of branching or lateral roots. Root hairs are elongations of a subset of root epidermal cells. They develop on both the primary and lateral roots. They have a variety of functions for the plant including providing an increased surface area for nutrient or water uptake, as well as sensing the external environment and gravity.
植物根包含主根及一些支根。根毛是根表皮細胞的部分延伸，它們在主根及支根上都有。根毛具多種功能包括提供更多表面積以吸收營養或水，也可感受外界環境及地心引力。



Root morphology. i = leaves/cotyledons, ii = hypocotyl, iii = lateral roots, iv = primary root, v = root hairs.
根的形態。i = 葉/子葉，ii = 下胚軸，iii = 支根，iv = 主根，v = 根毛

You have been provided with 5 agar plates with 5 different *Arabidopsis thaliana* genetic lines.
五個培養基分別有五種阿拉伯芥基因型植株

- Line T is a wild-type line 植株型 T 為野生型
- Lines U, V, W & X have abnormal root or root hair development/morphology
  植株型 U, V, W & X 具有不正常的根或根毛之發育/形態

1. Familiarise yourself with wild-type root and root hair morphology by carefully removing a seedling from one of the plants on plate T and studying it under the dissecting microscope.
   先從培養基 T 中，小心取出一棵小苗，以便熟悉野生型的根及根毛的形態，並在解剖顯微鏡下觀察其構造。
2. Mount a section of the root in water on a microscope slide, add a coverslip and observe the density and length of the root hairs under the compound microscope.
   取一小段根置於載玻片上，加水，蓋上蓋玻片，在複式顯微鏡下觀察根毛密度及長度。

Task 7a

Analyse lines U, V, W & X by following the same procedure. Complete the table with ONE code that best describes the phenotype that you observe. You can use each phenotype more than once.
再用相同的操作方法，分析 U, V, W & X 植株，描述所觀察到的表現型。將適當編號填入表格中，每個表現型可重複使用。

Root phenotype codes 根的表現型編號

Root phenotype 根的表現型	**Code** 編號
Short root hairs 短根毛	1
No root hairs 無根毛	2
Longer root hairs 較長根毛	3
Fewer root hairs 較少根毛	4
More root hairs 較多根毛	5
Shorter lateral roots 較短支根	6
Longer lateral roots 較長支根	7
No lateral roots 沒有支根	8

*Root phenotypes **(6 marks)** 根的表現型 **(6 分)***

Specimen 樣本	**Root development phenotype code** 根發育的表現型編號
U	
V	
W	
X	

Task 7b

Observe the wild type seedling (T) in the root hair zone. Choose ONE statement that best describes the pattern of the epidermal cells that are root hairs. Mark this statement with a cross (X) in the table below.

觀察野生型小苗 (T) 的根毛區，選擇一個最適當的敘述來描述具根毛的表皮細胞之型式。在下表中，用 (X) 標示最適當的敘述。

(2 marks) 2 分
(2 marks)

Root hair formation pattern 根毛形成的型式	X the correct statement 用 X 標示正確敘述
Root hairs are in rings of rows of epidermal cells. 根毛是一圈圈成行的表皮細胞	
Root hairs form from random cells. 根毛形成自任意的細胞	
Root hairs are in files or columns of epidermal cells. 根毛在表皮細胞上呈縱列	
Root hairs form a checkerboard pattern on the epidermis. 根毛在表皮上形成一棋盤狀	

Interpretation of your results 解釋結果
Plants have two copies of each gene (one on each chromosome from each parent). The wild type and mutant plants we have given you contain the same copy of each gene on both chromosomes (homozygous). If a plant contains two different copies (alleles) of a gene it is heterozygous.
Root hairs develop from surface (epidermal) cells but not all epidermal cells develop root hairs. One gene (called *WEREWOLF*) enables a root hair to develop, and other genes determine how long root hairs grow.
植物的每個基因有兩個複本，分別位在來自兩親本的對應染色體上。在此所提供的野生型和突變植株包含的基因屬同型合子 (homozygous)。若對偶基因不同則稱為異型合子。根毛從一部分表皮細胞 (非全部) 發育而來。*WEREWOLF*基因能使根毛發育，而其他基因則決定根毛可長多長。

Task 8a
If a plant was homozygous for a knockout mutation in the *WEREWOLF* gene (*werewolf*) AND also homozygous for another knockout mutation that usually caused extra-long root hairs, what type of root hairs would you expect to see on the plant? Mark the ONE correct answer with a cross (X) in the table.
若一個植物株為*WEREWOLF*基因剔除突變之同型合子(*werewolf*) 且同時也是造成根毛特別長的另一個剔除突變之同型合子，請問其根毛應該會是哪種型式? 在下表中，用 (X) 標示最適當的敘述。

(0.5 marks) (0.5 分)

Root hair phenotype 根毛表現型	X the correct statement 用 X 標示正確敘述
Normal length root hairs 正常根毛長度	
No root hairs 無根毛	
Extra long root hairs 根毛特別長	
Long root hairs 長根毛	

Task 8b

Complete the Punnet square for the expected genotypes of a cross between a *WEREWOLF* wild-type (BB) plant and a *werewolf* recessive homozygote (bb).
填完以下的棋盤方格 - *WEREWOLF* 野生型 (BB) 及 *werewolf* (bb) 雜交的基因型

(0.5 marks) (0.5 分)

blank 空白	Wild-type 野生型	Allele 1	Allele 2
Recessive homozygote 隱性的同型合子	*blank 空白*		
Allele 1			
Allele 2			

Two other plants were crossed and the progeny had the phenotypes shown in the table.
拿其他兩種植物來雜交，其子代的表現型如下表所示。

Phenotype 表現型	**Number of progeny** 子代數
Normal roots 正常根	24
No root hairs 無根毛	8

Fill in the table about this cross. 在表中填入有關此雜交方式的問題

(1.5 marks) (1.5 分)

What were the genotypes of the two plants that were crossed (in terms of B or b)? 這兩個拿來雜交的植物株之基因型為? (以 B or b 表示)	?? x ??
How many progeny have the genotype Bb? 多少子代的基因型為Bb?	
How many progeny have the genotype BB? 多少子代的基因型為 BB?	