25th INTERNATIONAL BIOLOGY OLYMPIAD

5 – 13 July, 2014

INDONESIA

PRACTICAL TEST 2

PLANT ANATOMY AND PHYSIOLOGY

植物解剖及生理學

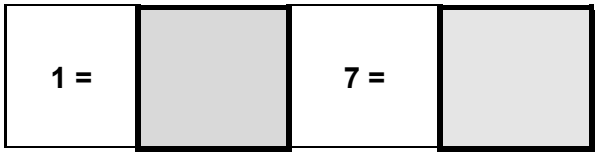
ANSWER SHEET 答案紙

Total points: **96**

Duration: 90 minutes

COUNTRY:	.....
STUDENT:	.....

The answers have to be given either with a tick (√) or with Arabic numbers. The numbers "1" and "7" can look very similar in handwriting. To make sure that those two numbers can be well distinguished by the IBO staff, please write them as you normally would into the following box.
答案必須是打勾(√)或阿拉伯數字，其中"1" 及 "7"的寫法很相似，為方便閱卷，請在下面空格中寫下你慣用的寫法。



Task 1: Determination of plant pigment (*36 points*)

第一大題: **植物色素分析**

TLC plate photograph (**4 points**). 色層分析板 (4 分)

Q 1.1 (12 points)

Spot	R*f* values of four major pigments (precision: two places after the decimal point) (@ ***2 point***) 四種主要色素的 R*f* 值(寫至小數點下兩位；每格 2 分)	Pigment number from Table 1 (@ ***1 point***) 對應表 1 的色素編號(每格 1 分)
1		
2		
3		
4		

Q. 1.2 (4 points)

Statement敘述	True 真	False 偽
A		
B		
C		
D		

Q 1.3 (10 points)

Extract 萃取液	A 吸光值		Total Chlorophyll 葉綠素總濃度(mg/L)	Chlorophyll a 葉綠素 a 濃度 (mg/L)	Chlorophyll b 葉綠素 b 濃度 (mg/L)
	649 (nm)	665 (nm)			
C					
D					

Q 1.4 (2 points)

Extract 萃取液	Ratio of chlorophyll 葉綠素比值
C	
D	

Q 1.5 **(4 points)**

Statement敘述	True 真	False 偽
A		
B		
C		
D		

Task 2: Determination of starch content in root extract (21 *points*)

第二大題: 植物根部萃取物的澱粉含量(21 分)

Q 2.1 **(1 point)**

Starch 澱粉溶液最終濃度 [ppm]	100
Starch solution 澱粉溶液 (µL)	
H_2O 水 (µL)	

Q 2.2 **(8 points)**

Sample 樣本	Absorbance 吸光值 (580 nm)
Starch 250 ppm (C4)	
Starch 100 ppm (C6)	
Sample C7	
Sample C8	

Q 2.3 (4 points)

a: ……

Q 2.4 (4 points)

Sample 樣本	Concentration 濃度 (ppm)
C7	
C8	

Q 2.5 (4 points)

Statement敘述	**True 真**	**False 偽**
A		
B		
C		
D		

Task 3. Observation of structural adaptation in plants (39 Points)

Q 3.1 (6 points @ 0.5 point)

Tissue type 組織類型	**Presence 有/無**		
Specimen 樣本	**X**	**Y**	**Z**
Cortex 皮層			
a. sclerenchyma 厚壁組織			
Endodermis 內皮			
Xylem 木質部			
a. primary xylem 初生木質部			
b. secondary xylem 次生木質部			

Q. 3.2 (9 points)

Specimen**樣本**	Diagram (number) 圖示代號
X	
Y	
Z	

Q 3.3 (9 points)

Specimen **樣本**	No aerenchyma 沒有通氣組織	Lysigenous* 溶解型	Schizogenous** 離生型
X			
Y			
Z			

* This type of intercellular space arises through dissolution of entire cells.
溶解型是藉由將該空間的所有細胞溶解而成之細胞間隙

** This type of intercellular space arises through separation of cell walls from each other along more or less extended areas of their contact.
離生型是藉由將細胞壁分開並擴增空間而成之細胞間隙

Q 3.4 (9 points)

Organ 器官	Monocotyledonous 單子葉植物			Dicotyledonous 雙子葉植物		
	Root 根	Stem 莖	Leaf 葉	Root 根	Stem 莖	Leaf 葉
X						
Y						
Z						

Q 3.5 (6 points)

Specimen樣本	Control 對照組	Flooding 淹水
X		
Y		
Z		

End of the Practical Exam 本實作結束!

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PLANT ANATOMY AND PHYSIOLOGY

植物解剖及生理學

Total points: **96**

Duration: 90 minutes

COUNTRY:	.....
STUDENT:	.....

Dear Participants

- This test consist of three tasks,

  本實作包括三大題

  Task 1: Determination of plant pigment (*36 points*)

  第一大題: 植物色素分析

  Part A: Qualitative identification of pigments by TLC

  Part A: 以色層分析判斷色素的種類(定性)

  Part B: Quantitative measurement of pigments by using spectrophotometer

  Part B: 利用光電比色器量測色素的量(定量)

  Task 2: Determination of starch in plant extract (*21 points*)

  第二大題: 植物澱粉萃取

  Task 3: Observation of structural adaptation in plants (*39 points*)

  第三大題:植物適應構造之觀察

- Please write your country name and student code in the box on title page.
  請在第一頁空格中寫下國家名稱及學生代碼
- **You have to write down your results and answers in the Answer Sheet. Answers written in the Question Paper will not be evaluated.**

  將答案寫於答案卷上，試題卷上的答案不計分!
- Write your answers legibly in ink.

  用原子筆清楚作答
- Please 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 hand immediately.**

  檢查所有列出的材料及器材，若有缺少，立刻舉手。
- **Safety: Always wear goggle, mask and gloves for TLC experiment (Part A).**
  安全注意: 操作色層分析(Part A)時，應配戴護目鏡、口罩及手套。
- Stop answering and put down your pen **immediately** when the bell rings.

  鈴響時，立即停止作答並放下筆。
- At the end of the test, place the **Answer Sheet** and **Question Paper** in the envelope provided. Our Assistants will collect the envelope from you.

  最後將答案卷及試題卷放入信封袋中，監試人員會來收卷。

Materials and Equipment 材料及器材

Task 1 第一大題

Plant material: 植物材料

- 2 x 500 µL of leaf ethanol-extract labeled A and B (in 1.5 mL microtube)
  2 種 500 µL 葉的酒精萃取液，分別置於標示 A 及 B 的 1.5 mL 小試管中
- 2 x 1000 µL of leaf ethanol-extract labeled C and D (in 1.5 mL microtube)
  2 種 1000 µL 葉的酒精萃取液，分別置於標示 C 及 D 的 1.5 mL 小試管中

Solution and reagents: 溶液及試劑

- 200 mL of hexane:acetone mixture
  200 mL of hexane:acetone 色層分析展開液
- 25 mL of alcohol (EtOH)
  25 mL of 酒精 (EtOH)

Technical material 器材

- 1 TLC tank 薄層色層分析缸 1 個
- 1 Micropipette 20-200 µL and yellow tips (1 box) 20-200 µL 微量分注器及黃色吸管尖
- 1 Micropipette 100-1000 µL and blue tips (1 box) 100-1000 µL 微量分注器及藍色吸管尖
- 2 Cuvettes 光電比色計小管 2 支
- 1 Cuvette rack 光電比色計小管架 1 個
- 2 Falcon tubes (15 mL) 15 mL 小試管 2 支
- 1 Timer 計時器 1 個
- 1 TLC plate (10 x 20 cm^2) 薄層色層分析板
- 1 Filter paper (20 x 20 cm^2) 濾紙
- 1 Forceps 鑷子 1 支
- 1 Pencil and sharpener 鉛筆及削鉛筆機 1 組
- 1 Ruler 尺 1 支
- 1 Calculator 計算機 1 台
- 1 Pair of gloves 手套 1 雙
- 1 Goggle 護目鏡 1 個
- 1 Mask 口罩 1 個
- 2 Cuvette label 光電比色計小管用的標籤紙 2 個

Task 2 第二大題

Plant material: 植物材料

- 2 x 1000 µL of root extracts labeled C7 and C8 (in 1.5 mL microtube)
  2 種 1000 µL 根萃取液，分別置於標示 C7 及 C8 的 1.5 mL 小試管中

Solution and reagents: 溶液及試劑

- 1500 µL of 250 ppm starch stock 250 ppm 澱粉濃縮溶液 1500 µL
- A bottle of I_2KI reagent 一瓶碘液
- A bottle of H_20 一瓶水

Technical material: 器材

- 1 Micropipette 100-1000 µL and blue tips (1 box) 100-1000 µL 微量分注器及藍色吸管尖
- 4 Cuvettes 光電比色小管 4 支
- 2 Microtubes(Eppendorf) (1.5 mL) 15 mL 小試管 2 支
- 1 Timer 計時器 1 個
- 1 Pair of gloves 手套 1 雙
- 1 Calculator 計算機 1 台
- 4 Cuvette labels 光電比色小管用的標簽紙 4 個

Task 3 第三大題

Plant material: 植物材料

- 3 x plant specimens labeled X, Y and Z (in Falcon 15 mL)
  三種植物分別置於標示為 X, Y and Z 的小試管中

Solution and reagents: 溶液及試劑

- A bottle of H_2O 一瓶水
- A bottle of Aniline Sulphate– stain lignin – yellow color 一瓶 Aniline Sulphate 試劑 (將木質素染成黃色)
- A bottle of Sudan III – stain lipid, i.e. suberin – red color 一瓶蘇丹三號試劑 (將脂質染成紅色)

Technical material 器材

- 1 microscope 顯微鏡 1 台
- 2 razor blades 刀片 2 支
- 6 microscope slides 載玻片 6 片
- 12 cover slips 蓋玻片 12 片
- 1 forceps 鑷子 1 支
- 1 dissecting needle 解剖針 1 支
- Tissue paper 衛生紙

Note : Use given materials properly! No additional materials will be provided in this experiment. 注意: 適度使用材料，用完後不再提供!

Task 1: Determination of plant pigment
第一大題: 植物色素分析

Several plants are resistant to flooding stress by various physiological and morphological defense mechanisms. Prolonged levels of water stress may cause several physiological symptoms resulting in death.
許多植物可藉由不同的生理或形態防禦機制來抵抗淹水逆境。長期處於水逆境會導致許多會造成死亡的生理徵狀。

In this task, plants were grown for 2 weeks with 12/12 h (light/dark) photoperiod and exposed either to flooding treatment or normal watering as control.
本大題中，植物在 12/12 小時光/暗之光週期下生長 2 週，實驗組給予淹水處理、對照組則正常澆水。

To analyze the physiological conditions, leaf samples of stressed and normal plants were extracted and analyzed for pigments. These include qualitative observation by Thin Layer Chromatography (TLC) and quantitative measurement by spectrophotometry analysis.
為分析其生理狀況，分別取下逆境及正常生長的植物葉片，萃取其色素，以薄層色層分析法來分辨色素種類(定性)、以光電比色計來量測其色素量(定量)。

Part A Qualitative identification of pigments by TLC (*20 points*)
Part A: 以薄層色層分析判斷色素的種類(定性)

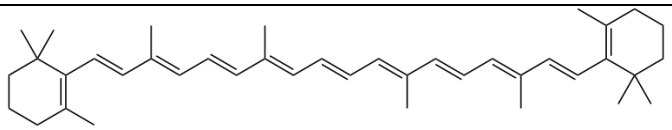
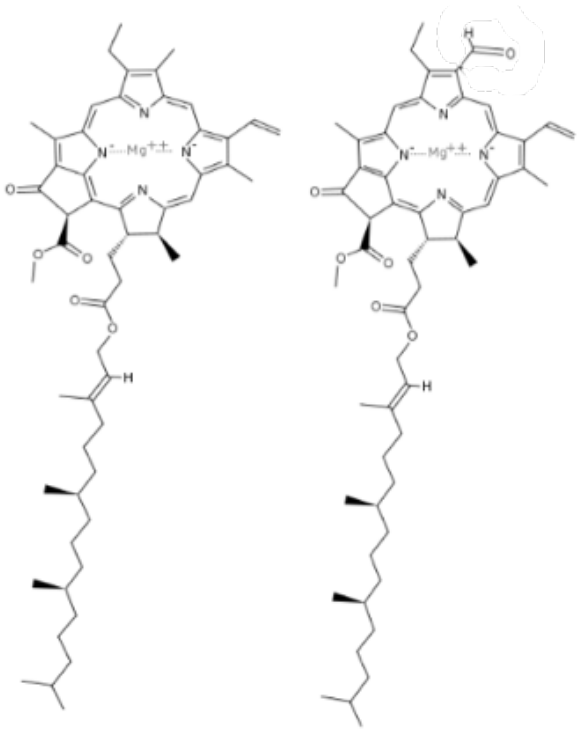
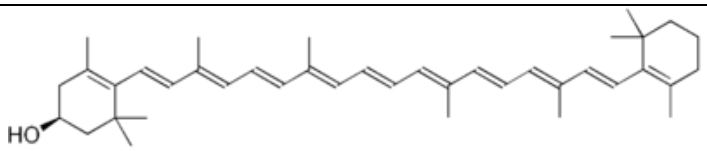
You will be provided with one extract from treated-plants and the other from control leaves, arbitrarily labeled A and B run on TLC plate together. Prepare the task by:
將所提供的實驗組及對照組葉片萃取液(分別標示為 A and B)同時進行色層分析，操作方法如下。

- Attach the filter paper provided to one of the inside TLC tank walls.
  將濾紙緊靠在薄層色層分析缸的一側。
- Add 200 mL of hexane:acetone (7:3) solution to the TLC tank, and close the lid.
  在薄層色層分析缸加入 200 mL of hexane:acetone 色層分析展開液，並蓋上蓋子。
- Use a pencil to lightly mark the edge of TLC plate 2 cm from bottom, top, left and right edges. Draw lines one on the bottom and one on the top of the TLC plate. Indicate A and B on the bottom line.
  用鉛筆在薄層色層分析板的上、下、左、右四邊分別輕輕畫上 2 公分邊界。並在下底線上標上 A、B 兩點。
- Micropipette 100 µL of Extract A and discharge the extract on the bottom line of TLC plate and repeat with Extract B. Make sure you concentrate the extract on one spot.
  以微量分注器取 100 µL 的 A 萃取液，緩慢點在下底線的 A 點上，並盡量集中在一點上；重複操作 B 萃取液於 B 點上。

- Air-dry the plate for 5 minutes then place the plate into the TLC tank.
  色層分析板風乾 5 分鐘，然後放入色層分析缸中。
- As the eluent reaches the top line, remove the TLC plate immediately from the tank. Mark the end of the solvent front.
  當移動最快的色素到達上方邊線時，立刻取出薄層色層分析板，並標記展開液到達的位置。
- Remove the TLC plate and place it on the sheet provided with your student code. Raise your hand. An assistant will take a photograph of your TLC plate for grading (***4 points***).
  將薄層色層分析板放在寫有你的學生代碼的紙上，舉手請監試人員將拍照以便打分數(4 分)。

Question 1.1 Calculate the R*f* values which are a ratio between distance of center of the spot to start line and distance of eluent from start to stop line and determine tentative name of pigments. Identify the pigments using the Table below (***12 points***). 計算每個色素點的 R*f* 值(色素移動距離/展開液移動距離)，其中色素點距離是自色素原點量測至色素帶的最高點。利用下表來判斷色素種類。(12 分)

Table 1. List of 4 major pigments to be identified 四種主要色素

No.	**Pigment** 色素	**Chemical structure** 化學結構
1	β-carotene β-胡蘿蔔素	
2	chlorophyll a 葉綠素 a	chlorophyll a chlorophyll b 
3	chlorophyll b 葉綠素 b	
4	xanthophylls 葉黃素	

Question 1.2 Indicate with a tick (√) if each of the following statements is True or False **(*4 points*). 判斷下列敘述之真偽，在適當空格中打勾(√)**。(4 分)

A. R*f* values of chloropyll a and chlorophyll b are different due to their molecular weights.
葉綠素 a 及 b 的 R*f* 值不相同，是因為其分子量不同。

B. R*f* values of chlorophylls and carotenoids are different due to their polarity.
葉綠素 a 及胡蘿蔔素的 R*f* 值不相同，是因為其極性不同。

C. Flooding stress affects the concentration of chlorophyll b.
淹水逆境會影響葉綠素 b 的濃度。

D. Plant A was grown in flooded conditions.
生長在淹水逆境下的是植物 A。

Part B Quantitative measurement of pigments using spectrophotometer (*16 points*)

Part B: 利用光電比色器量測色素的量(定量) (16分)

Quantitative measurement using spectrophotometer is essential to determine content of pigment in control and flooded plants. In this task, you will be given leaf extracts C and D.
對照組及淹水植物(實驗組)的色素定量須利用光電比色器來測量。本大題則提供葉片萃取液任意標示為 C 及 D。

1. Transfer 1 mL of each extract into two separate 15 mL Falcon tubes.
   分別取 1 mL 的萃取液至兩個 15 mL 小試管。
2. Dilute each extract to 10 mL with alcohol and mix the solution well.
   每管再以酒精稀釋為 10 mL，並混合均勻。
3. Pipette the diluted Extract C into the cuvette and label with C and repeat for Extract D.
   吸取稀釋後的萃取液 C 至標示為 C 的光電比色小管中，重複操作萃取液 D。
4. **Raise your hand. Lab assistants will take your cuvettes to measure** absorbance (A) (λ = 649 and 665 nm) and provide you with the readings.
   舉手提示監試人員前來將你的兩管拿去測量兩種波長的吸光值(A) (λ = 649 and 665 nm)，他會給你數據。
5. Calculate the concentrations of chlorophyll a, b and total (mg/L) using the following equations (Wintermans & de Mots, 1965).
   利用以下的公式來計算葉綠素 a, b 的個別濃度及總濃度。

   Total chlorophyll (mg/L) = 20.0 (A_{649}) + 6.1 (A_{665})
   Chlorophyll a = – 5.76 (A_{649}) + 13.7 (A_{665})
   Chlorophyll b = 25.8 (A_{649}) – 7.7 (A_{665})

Question 1.3 Write down the absorbance values into the **Answer Sheet**. Calculate the content of total chlorophyll and chlorophylls a and b. (precision: two places after the decimal point) (***10 points***). 在答案紙上寫下各吸光值，計算葉綠素總濃度以及葉綠素 a, b 的個別濃度至**小數點後兩位**。(10 分)

Question 1.4 Calculate the ratios of chlorophyll a to b for Extracts C and D (precision: two places after the decimal point) (*2 points*). 分別計算萃取液 C 及 D 的葉綠素 a,b 比值(a/b 的數值)至小數點後兩位。(2 分)

Question 1.5 Indicate with a tick (√) whether each of the following statements is True or False (***4 points***). 判斷下列敘述之真偽，在適當空格中打勾(√)。(4 分)

A. Under the flooding stress, the degradation of chlorophyll b is higher than of chlorophyll a.
在淹水逆境下，葉綠素 b 的降解較葉綠素 a 多。

B. Photosynthetic rate in Plant C is higher than in Plant D.
植物 C 的光合作用速率較植物 D 高。

C. Flood-stress plant has a lower nitrogen absorption than under normal conditions.
淹水逆境植物對氮的吸收能力較正常狀態下的植物低。

D. Plant C was grown in flooded conditions.
植物 C 生長在淹水逆境下。

Task 2: Determination of starch content in root extract (*21 points*)

第二大題: 植物根部萃取物的澱粉含量

Under flooded conditions, the respiration of roots and microorganisms quickly deplete the remnant oxygen in the soil. As a result, flooded tissues will rely more heavily on anaerobic metabolic pathways. In this task, you will quantify the effect of the lack of oxygen on the recruitment of carbohydrate from starch reserves using spectrophotometry on extracts from flooded and control plants, arbitrarily labeled C7 and C8.
在淹水逆境下，根及微生物的呼吸會快速用完土壤中的氧氣。導致淹水的組織將大量依賴無氧呼吸代謝路徑。本大題中，你將利用光電比色器來測量淹水及對照植物之萃取液(任意標示為C7 及 C8)，來量化植物在缺氧時，將澱粉轉換為可利用之碳水化合物的作用。

Part 2.1 Measurements 測量

In order to quantify starch content using spectrophotometry, you will compare the absorbance of the Lugol-stained plant extracts with the absorbance measured for dilutions of 250 ppm and 100 ppm starch.
為使用光電比色器來量化澱粉含量，你將植物萃取液以碘液(Lugol-stain)染色後的吸光值，分別與 250 ppm and 100 ppm 的澱粉溶液染色後的吸光值做比較。

Question 2.1 You are provided with a stock solution of 250 ppm starch. Prepare an additional 1000 µL stock solution of 100 ppm. Begin by indicating the required volumes of the 250 ppm starch stock solution and H_2O in the **Answer Sheet.** Pipette these volumes into a fresh microtube and mix well **(1 point)**. 由所提供的 250 ppm 澱粉濃縮溶液，製備稀釋為 100 ppm 的澱粉溶液 1000 µL，在答案紙上填入稀釋時所需的 250 ppm 原液的量以及需加入的水量。

Prepare now each of the four samples; starch dilutions of 250 ppm (C4), 100 ppm (C6), plant extracts (C7 and C8) as follows in cuvettes:
製備四個光電比色小管之樣本: 250 ppm 澱粉溶液(標示為 C4)、100 ppm 澱粉溶液(標示為 C6)、兩種植物萃取液(標示為 C7 and C8)。

1. Pick one of the provided labels and stick it to a cuvette.
   在光電比色小管貼上標籤
2. Pipette of 900 µL of the sample into the cuvette.
   吸取 900 µL 的樣本至光電比色小管中
3. Add 100 µL of Lugol (I_2KI) solution and mix well.
   加入 100 µL 碘液於管中，混合均勻
4. Incubate for 4 minutes at room temperature.
   在室溫下作用 4 分鐘

Raise your hand when your samples are ready. The assistant will then collect your samples for measurement and return a print out of the obtained absorbance.
當你的樣本處理好了，舉手通知監試人員，他會將你的樣本拿去測量，然後把數據列印給你。

Question 2.2 Fill the table of absorbance values in your **Answer Sheet** (***8 points***).

在答案紙的表格中填入吸光值。(8 分)

Part 2.2 Data analysis 數據分析

Under the assumption that absorbance is changing linearly with the starch concentration and is 0 for a starch concentration of 0 ppm, the relationship between starch concentration (x) and absorbance (y) is given by

$$y = ax$$

假設吸光值與澱粉濃度呈線性關係，且當澱粉濃度為 0 時，其吸光值為 0；其關係式如上所示，其中 x 為澱粉濃度，y 為吸光值。

Question 2.3 Use a linear regression approach to estimate the **slope (a)** of the above equation as 以線性相關法來估算上面公式的斜率**(**a)，如下:

$$a = \frac{\Sigma xy}{\Sigma x^2}$$

and report the obtained result with a precision of two places after the decimal point in the **Answer Sheet** (**4 points**). 然後計算數值**至小數點後兩位**，填入答案紙中。

Question 2.4 Use your estimate of the **slope** (**a**) to calculate starch concentrations (ppm) in Samples C7 and C8 (integer, ***4 points***).
利用你估算的斜率**(a)**來計算 C7 及 C8 兩樣本的澱粉濃度(ppm)，**取整數**。(4 分)

Question 2.5 Indicate with a tick (√) if each of the following statements is True or False based on your observation (***4 points***).
根據你的觀察，判斷下列敘述的真偽，並在答案紙的適當空格中打勾(√)。

A. After flooding, the concentration of sucrose and other soluble sugars increase in the roots.
淹水之後，根中的蔗糖及其他可溶性糖類的濃度上升。
B. Impaired carbon translocation facilitates starch accumulation in roots of flooded plants.
碳水化合物的運輸作用受損有助於澱粉在淹水植物根中的累積。
C. Alcoholic fermentation in the root increases as a result of flooding.
根中的酒精發酵會因淹水而增加。
D. C7 was grown in flooded conditions.
C7植物是生長在淹水情況下。

Task 3. Observation of structural adaptation in plants (*39 points*)
第三大題: 植物適應構造之觀察

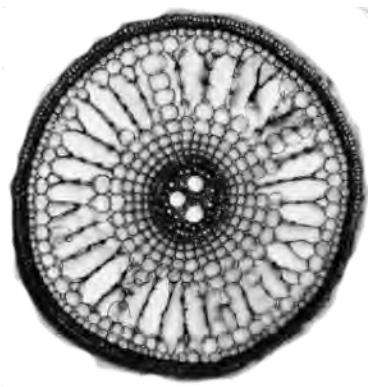
Anatomical and morphological changes in root and shoots usually occur as structural adaption to waterlogged soils. The stress brought on by low O_2 concentrations in flooded soils leads to the formation of aerenchyma in some tolerant plant species, a process that can occur in roots, nodules, rhizomes, stems and submerged leaves.
植物在適應淹水的土壤時，通常在根與莖的解剖構造及形態上發生改變。某些耐淹水的植物在淹水缺氧時，會有通氣組織的形成，此現象通常發生在根、根瘤、根莖、莖及沉水的葉片中。

Find three specimens in Falcon Tubes **X, Y,** and **Z**. These specimens were obtained from plants developed in two different soil conditions.
在小試管 **X, Y,** and **Z** 中，共有三種標本，他們是來自兩種土壤狀況。

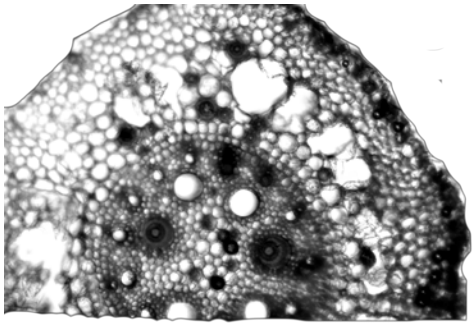
a. Make a cross section of each specimen and stain with staining solutions provided.
   分別將標本作徒手切片，取得橫切面並以所提供的染料來染色。
b. Observe the structure of stained specimens under the light microscope.
   用光學顯微鏡觀察染色後的標本切片。

Question 3.1 Observe the sections carefully and complete the table on the **Answer Sheet** using symbols: "+" for presence, "-" for absence. ***(6 points @0.5)***
仔細觀察切片並在答案紙的表格中**以"+"表示" 有" ；"-"表示" 無"** 。(6分，每小格0.5分)

Question 3.2 Indicate the diagram number corresponding to the specimen (***9 points***).
由以下所提供之圖示代號來標示所對應的標本。(9 分)

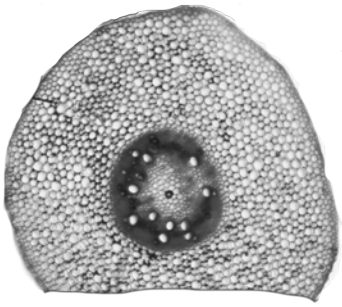


1

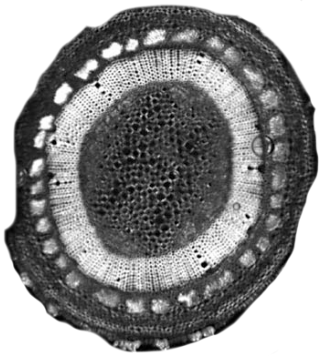


2

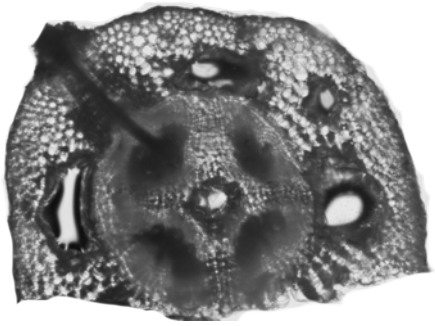
3



4

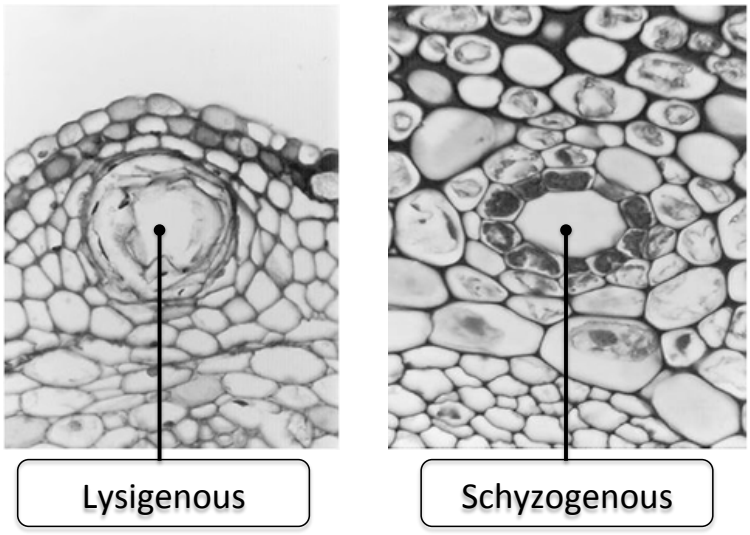


5



6

Question 3.3 Indicate with a tick (√) the type of aerenchyma of each specimen (***9 points***)
標出每個標本的通氣組織類型。下圖所示: Lysigenous (溶解型)；Schyzogenous(離生型)



Question 3.4 Indicate with a tick (√) link the corresponding specimens with particular plant organ(s) based on your observations (***9 points***).
標示出每個標本所對應的特定器官，並在適當空格中打勾(√)。(9分)

Question 3.5 Indicate with a tick (√) for each of the corresponding specimen with their growth condition (***6 points***)
標示出每個標本所對應的生長狀況，並在適當空格中打勾(√)。(6分)

End of the Practical Exam

本實作結束!