INTERNATIONAL BIOLOGY OLYMPIAD e. V. IBO

Student name:	Student code:	Country:

28th International Biology Olympiad

July 23-30, 2017
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

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

You can perform the tasks in any order but note that task 6a** includes a 1 hour incubation step:

Question 1: Plant taxonomy
Task 1 – Identify plant species samples and the ploidy of their tissue. (4 Marks)
Task 2 – Identify flower families and dissect flowers to identify carpel structure. (7.5 Marks)
Task 3 – Prepare stem sections and identify vascular structure and stem organisation. (9 Marks)
Task 4 – Determine the phylogeny of species used in tasks 1-3. (3 Marks)
Question 2: Floral morphology
Task 5 – Dissect flowers to determine floral part identity and flower organisation. (10 Marks)
Question 3: Seed and embryo development
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
Question 4: Root morphology
Task 7 – Observe and determine types of root hair and root development in seedlings. (8 Marks)
Task 8 – Determine the manner of inheritance of root development genes. (2.5 Marks)

This is a test of sample handling and manipulation, fine dissection skills, observation and reasoning.
Good luck!

Important Information:

- Please remember to write your name, your student code and your country in the given boxes.
- Write your answers in this question booklet. Only the answers given on this question booklet will be evaluated.
- Make sure that you have received all the materials and equipment listed. If any of these items are missing, please raise your flag immediately.
- During experiments, ensure that you handle equipment and samples properly. Any spilled solutions, samples or equipment damaged by you will not be replenished.
- Stop answering and put down your pen immediately when the whistle sounds at the end of the exam.
- Leave the question booklet on your desk at the end of the exam.
- No paper, materials or equipment should be taken out of the laboratory.

MATERIAL AND EQUIPMENT

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.

Required for all questions:

- Stereo (Dissection) Microscope
- Compound microscope
- Hand Lens
- Straight teasing needles
- Curved fine point forceps
- Fine point forceps
- Small scissors
- Waste beakers
- Permanent marker pens
- Roll of white tissue
- Powder free disposable nitrile gloves
- Lab Coat
- Safety Goggles
- 2 blank paper sheets for notes
- Flag to call the lab assistant
- **An English translation of this paper is available upon request.**

Question 1: Plant taxonomy

Plant material

- 7 plant specimens labelled A, B, C, D, E, I, and J
- 5 flowers labelled E, F, G, H,and I (specimens from the same species (E and I) are used in tasks 2 AND 3)
- 3 microscope slides of plant sections labelled K, L, and M

Solutions and reagents

- 10ml Solution A (Toluidine blue stain)
- 10ml Solution B (Distilled water)

Technical material

- Plastic teat pipettes
- Safety razor blades
- 12 microscope slides and 12 cover slips (total for all tasks)
- Disposable jars for used slides/razor blades
- 2 petri dishes
- Timer

Question 2: Floral morphology

Plant material

- 3 tubes containing inflorescence (flowering stem) tips with flowers from 3 different *Arabidopsis thaliana* genetic lines. Labelled O, P, and Q

Question 3: Seed and embryo development

Plant material

- 2 tubes containing inflorescences (flowering stems) from 2 different *Arabidopsis thaliana* genetic lines that are developing seeds. Labelled R, and S.

Solutions

- 1.5 ml Solution C (Hoyer's solution (please note this is an irritant chemical solution))

Technical material

- Electrical tape (yellow)
- Hypodermic needles for dissection
- Safety razor blades
- 12 microscope slides and 12 cover slips (total for all tasks)
- Disposable jars for used slides/razor blades
- Double sided sticky tape (white)
- Timer
- 15 cm ruler

Question 4: Root morphology

Plant material

- 5 agar plates of seedlings: 5 different *Arabidopsis thaliana* genetic lines labelled T, U, V, W, and X

Technical material

- 12 microscope slides and 12 cover slips (total for all tasks)

QUESTION 1: PLANT TAXONOMY

(23.5 marks)

Introduction
As plants have evolved they have acquired morphological characteristics related to the function that they provide. Species can be identified by their characteristics.

Task 1
Species ploidy identification
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.

Specimen table key

Species type	**Species code**
Angiosperm	1
Moss	2
Conifer	3
Fern	4

Ploidy level	**Ploidy code**
Haploid: if the tissues or cells in the specimen are from all or part of one type of gametophyte only.	n
Diploid: if the tissues or cells in the specimen are from all or part of one sporophyte generation only.	2n
Tissues or cells from all or part of two sporophyte generations.	2n+2n
Tissues or cells from all or part of one sporophyte generation and two types of gametophytes.	2n+n+n
Tissues or cells in the specimen are from all or part of one sporophyte generation and one type of gametophyte.	2n+n

Specimen table ***(4 marks)***

Specimen	**Species code**	**Ploidy level code**
A		
B		
C		
D		

Task 2

Floral structure

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.

Flower specimen key

Family	**Family code**
Asteraceae	1
Brassicaceae	2
Lamiaceae	3
Fabaceae	4
Onagraceae (not in syllabus)	5
Magnoliaceae	6

Gynoecium position	**Gynoecium code**
Hypogynous flower (superior ovary)	7
Epigynous flower (inferior ovary)	8
Perigynous flower (neither superior or inferior ovary)	9

Carpel structure	**Carpel code**
Single ovary of one carpel only	10
Ovary of fused carpels	11
Many separate carpels (compound flower)	12

Flower specimen table ***(7.5 marks)***

Specimen	Family code	Gynoecium position code	Carpel structure code
E			
F			
G			
H			
I			

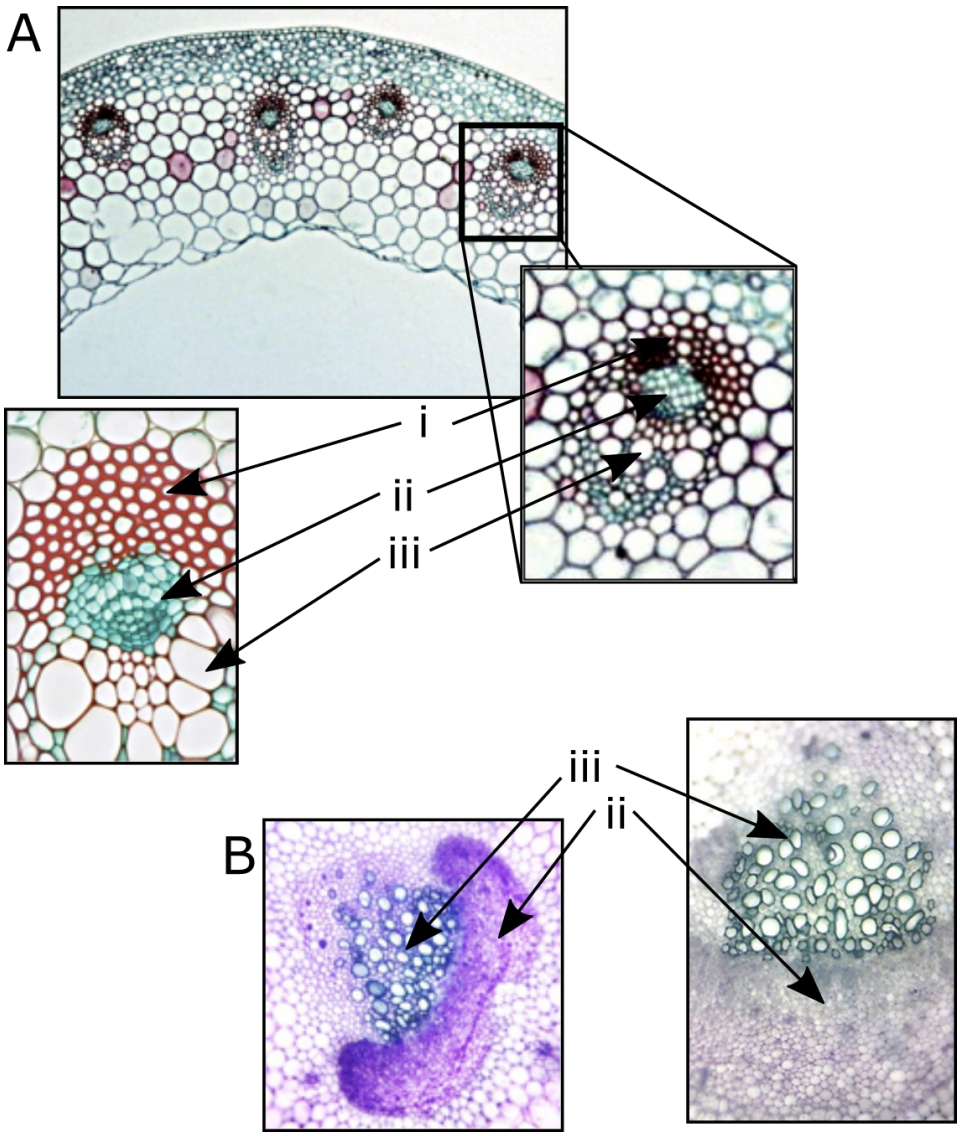
Task 3

Introduction

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.

Feature	Xylem vessels	Phloem	Sclerenchyma fibres
Function	Transport of water and mineral ions Structural support	Transport of dissolved carbohydrates	Structural support
Structure	Hollow tubes made of many connected cells	Hollow tubes made of many connected cells	Separate cells joined together to form fibres
Dead/alive	Dead cells	Sieve tube elements kept alive by connections to companion cells	Dead cells
Cell wall	Thickened and possess lignin	No special modifications (plasmodesmata between sieve tube element and companion cells, also sieve plates at the end of cells)	Thickened and possess lignin
Cell contents	Completely hollow – some perforated or slitted end plates of cells remain	Hollow centre with thin layer of cytoplasm around the edge. Sieve plates at the end of each cell.	Hollow dead cells

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).



Transverse sections of stems. i = sclerenchyma fibres, ii = phloem, iii = xylem. A = buttercup, B = celery.

Vascular tissues of the stem are organized in continuous strands in several ways, depending on the spatial location of the cells:

- 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.
- In **collateral** vascular bundle organization the phloem is located towards the outside and the xylem is located towards the inside.
- In **concentric amphivasal** vascular bundle organization the xylem encloses the phloem.
- In **concentric amphicribral** vascular bundle organization the phloem surrounds the xylem.
- In **monocotyledons (monocots)**, there are **scattered** vascular bundles, dispered over the stem, although more towards the periphery
- In **dicotyledons (dicots)**, there are is a **ring** of vascular bundles, arranged in the stem to form a cylinder
- In **woody** species, thickening of the stem occurs, visible as **annual ring** formation.

Stem rigidity is maintained either by turgor pressure inside cells, or secondary wall material (termed secondary thickening).

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.

1. Hold the stem of the plant in a vertical angle between the thumb and the index of your non-dominant hand.
2. Hold the razor with your dominant hand and start cutting transverse sections of the stem, as thin as possible.
3. In one petri dish, add a few drops of solution A (blue stain).
4. Place the transverse sections into the stain and stain for 3-5 minutes; staining time depends on the thickness of the section.
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.
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).

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.

Section specimen key

Vascular bundle type	**Type code**
Bicollateral	1
Collateral	2
Concentric amphivasal	3
Concentric amphicribral	4

Bundle organisation	Bundle code
Arranged in a ring	5
Scattered	6
Annual rings	7

Stem rigidity	Rigidity code
Turgor pressure	8
Secondary thickening	9

Section table ***(9 marks)***

Specimen	Vascular bundle type code	Vascular bundle organisation code	Provision of stem rigidity code
E			
I			
J			
K			
L			
M			

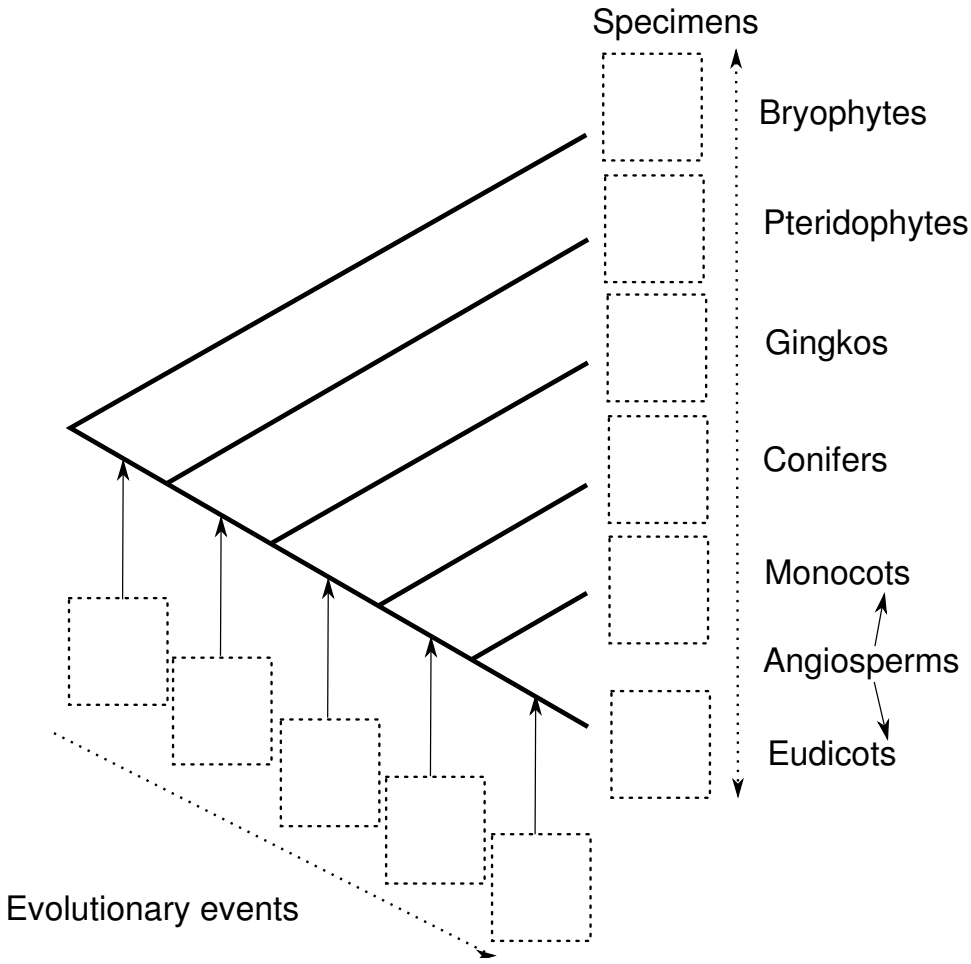
Task 4

Taxonomy

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.

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):

I. Development of first flowers
II. Development of first vasculature
III. Development of first seeds



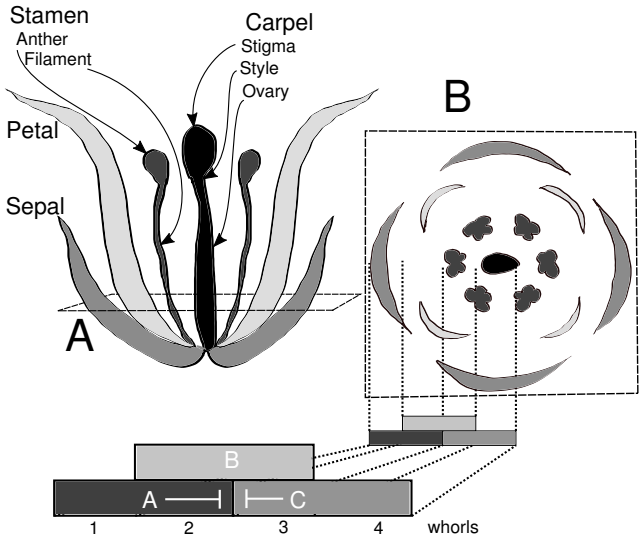
Taxonomic tree ***(3 points)***

QUESTION 2: FLORAL MORPHOLOGY

(10 marks)

Introduction

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.



- Expression of gene A specifies sepals.
- Expression of genes A and B together specifies petals.
- Expression of genes B and C together specifies stamens.
- Expression of gene C specifies carpels.
- Genes A and C repress expression of each other, thus are mutually exclusive.

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.

Organ identity codes

Organs present	**Organs code**
Sepals, petals, stamens and carpels	1
Sepals only	2
Stamens and carpels only	3
Sepals and stamens only	4
Sepals and petals only	5
Sepals and carpels only	6

Classes of gene activity	**Gene code**
A, B, C	7
A, B	8
A, C	9
B, C	10
A	11
B	12
C	13

Genotype	**Genotype code**
Wild-type	14
Mutant	15

Floral organ identity table ***(9 marks)***

Specimen	**Organ identity code**	**Homeotic gene activity code**	**Genotype code**
O			
P			
Q			

Task 5b

Complete the table below choosing ONE appropriate code for each answer. You can use each code more than once.

Phenotype codes

Phenotype	**Phenotype code**
Leaves in all four whorls	1
Sepals in all four whorls	2
Petals in all four whorls	3
Carpels in all four whorls	4
Stamens in all four whorls	5

(1 mark)

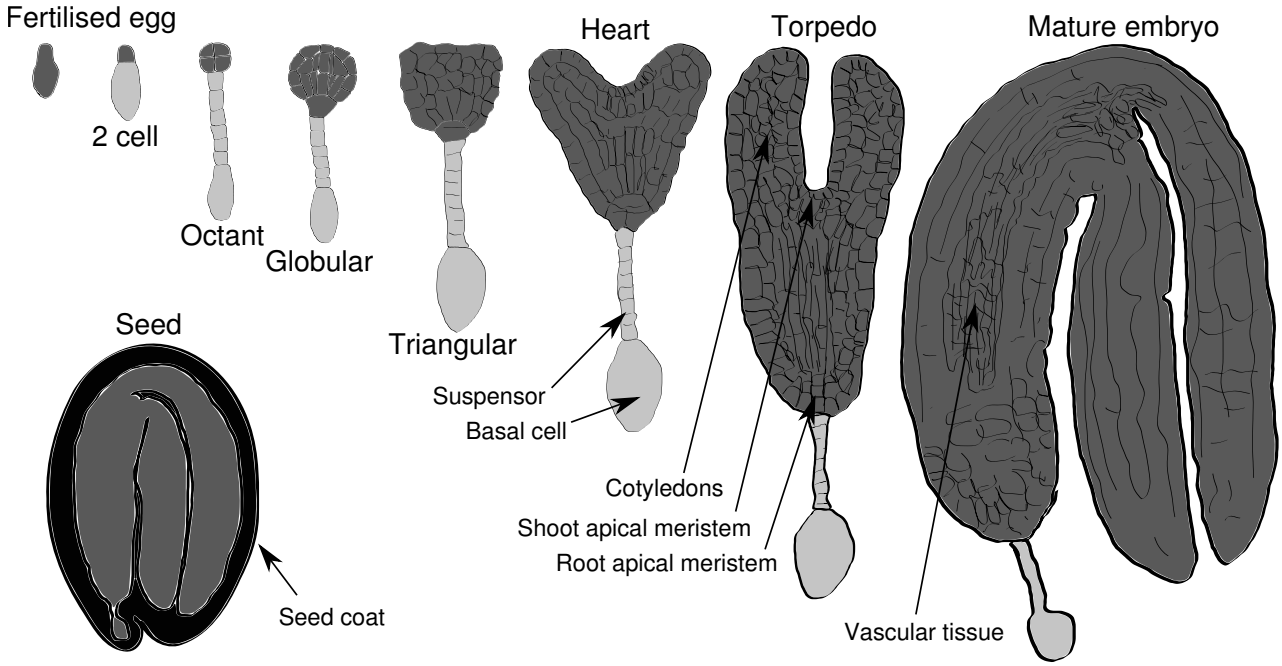
	Phenotype code
What is the flower phenotype in a homozygous knockout BC double mutant?	
What is the flower phenotype in a homozygous knockout ABC triple mutant?	

QUESTION 3: SEED AND EMBRYO DEVELOPMENT

(10 marks)

Introduction

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).



Arabidopsis embryo developemnt

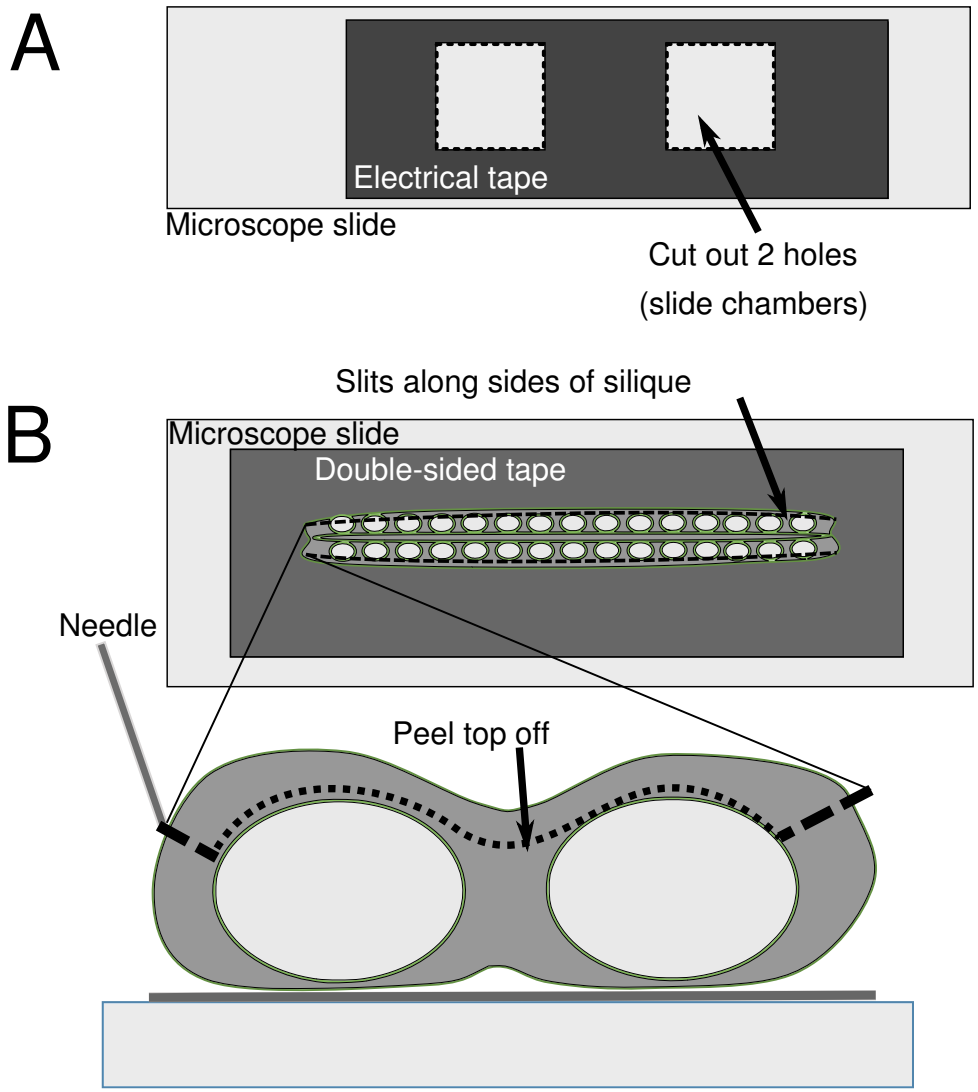
Silique age progression. i = youngest flowers, ii = older embryos and seeds.

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.*

Silique dissection procedure

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.
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.
3. Observing the slide under a dissecting microscope, record the number of seeds and the number of aborted seeds (if any) within the silique.
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.
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).
6. Observe the samples at high magnification using a compound microscope (e.g. you can see a heart-shaped embryo in this cleared seed).



Silique dissection

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.

Silique morphology ***(4 marks)***

Plant	Average number of seeds	% globular stage	% heart stage	% torpedo stage	% mature stage
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?

Complete the table below, choosing ONE code for each question.

Development codes

Seed defect	**Defect code**
Embryo pattern formation is disrupted	1
Seeds lack embryos	2
Seeds have multiple embryos	3
Seeds have enlarged embryos	4

Approximate frequencies of abnormal embryos	**Approximate frequency code**
0%-33%	5
34%-66%	6
67%-100%	7

Cause of embryo abnormality	**Cause code**
Double fertilisation	8
Development of a non-embryonic cell	9
Spontaneous	10

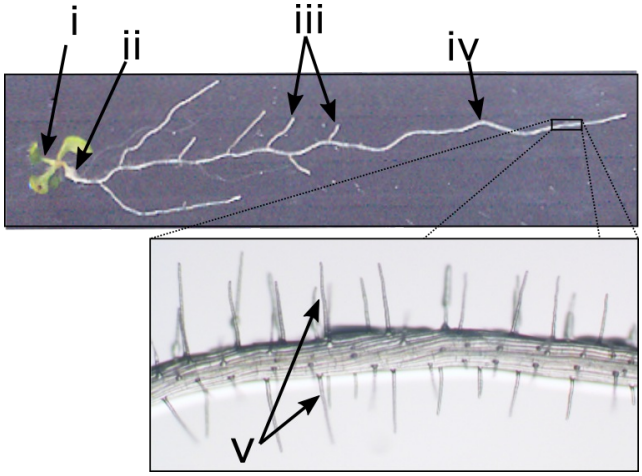
(6 marks)

	Which plant line has abnormal development? (R or S)	Defect code	Approximate frequency code	Cause code
Answer				

QUESTION 4: ROOT MORPHOLOGY

(10.5 marks)

Introduction
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.



Root morphology. i = leaves/cotyledons, ii = hypocotyl, iii = lateral roots, iv = primary root, v = root hairs.

You have been provided with 5 agar plates with 5 different *Arabidopsis thaliana* genetic lines.

- Line T is a wild-type line
- Lines U, V, W & X have abnormal root or root hair development/morphology

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.
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.

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.

Root phenotype codes

Root phenotype	**Code**
Short root hairs	1
No root hairs	2
Longer root hairs	3
Fewer root hairs	4
More root hairs	5
Shorter lateral roots	6
Longer lateral roots	7
No lateral roots	8

Root phenotypes ***(6 marks)***

Specimen	**Root development phenotype code**
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.

(2 marks)

Root hair formation pattern	X the correct statement
Root hairs are in rings of rows of epidermal cells.	
Root hairs form from random cells.	
Root hairs are in files or columns of epidermal cells.	
Root hairs form a checkerboard pattern on the epidermis.	

Interpretation of your results
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.

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.

(0.5 marks)

Root hair phenotype	X the correct statement
Normal length root hairs	
No root hairs	
Extra long root hairs	
Long root hairs	

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).

(0.5 marks)

blank	Wild-type	Allele 1	Allele 2
Recessive homozygote	*blank*		
Allele 1			
Allele 2			

Two other plants were crossed and the progeny had the phenotypes shown in the table.

Phenotype	**Number of progeny**
Normal roots	24
No root hairs	8

Fill in the table about this cross.

(1.5 marks)

What were the genotypes of the two plants that were crossed (in terms of B or b)?	?? x ??
How many progeny have the genotype Bb?	
How many progeny have the genotype BB?	