INTERNATIONAL BIOLOGY OLYMPIAD e. V. IBO

27th International Biology Olympiad

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Hanoi, Vietnam

Practical Exam 1

PLANT ANATOMY AND PHYSIOLOGY

Total points: 91
Duration: 90 minutes

DEAR PARTICIPANTS,

This exam consists of three experiments:

EXPERIMENT 1. LEAF PIGMENT ASSAY (30 points)

Task 1. Pigment quantitative determination by spectrophotometer

Task 2. Pigment qualitative determination by TLC

EXPERIMENT 2. PLANT ANATOMY (31 points)

Task 3. Observe the anatomical characters of four samples

Task 4. Make the data matrix and identify the position of each sample in a given phylogenetic tree

Task 5. Draw the detailed structures of that vascular bundle

EXPERIMENT 3. IDENTIFICATION OF PLANT SPECIES AND MAKE THE DATA MATRIX (30 points)

Task 6. Identify morphological and anatomical characters of five given floral samples

Task 7. Name samples PG-PK using a given dichotomous key

Task 8.Make the data matrix

A. Please remember to write your **Country** and **Student code** in the given box.
B. Write your answers in the separate **Answer Sheet**. Only the answers given in the **Answer Sheet will be evaluated**.
C. Make sure that you have received all the materials and equipment listed. If any of these items are missing, please raise the **Red card** within 10 minutes immediately.
D. During experiments, ensure to handle equipment properly. Any spilled solutions or broken equipment will not be replenished.
E. Stop answering and put down your pen immediately when the bell rings at the end of the exam. Enclose the **Answer Sheet**, **Question Paper**, and **Data printout** in the provided envelope.
F. No paper, materials or equipment should be taken out of the laboratory
G. Ensure to obtain spectrophotometer readings in Task 1 and to answer the questions that follow.

CAUTIONS: This experiment deals with materials that are fragile and sharp. Exercise care when handling these materials. Do not let them get in contact with your skin or clothes. Wear safety goggles to protect your eyes from splashes.

Good luck!!!

EQUIPMENT AND MATERIALS FOR 3 EXPERIMENTS

Experiment 1. Leaf pigment assay

Name	Quantity
Soybean leaf samples (sample A and sample B)	2 microcentrifuge tubes
Positive control	1 microcentrifuge tube
TLC plate with student code in a plastic bag	1 piece
Cuvettes	2 pieces
95% ethanol	40 mL in falcon tube
Ethanol for washing pipette	20 mL in falcon tube
Chromatography solvent (n-hexane : acetone = 7:3 in volume)	25 mL in TLC bottle
Mortars and pestles	2 pieces
Falcon rack	2 pieces
Funnels	2 pieces
Filter papers	2 pieces
Forceps	1 pairs
1 mL glass pipette	2 pieces
5 mL glass pipette	1 piece
Pipetting ball	1 piece
1.5 mL microcentrifuge tube	2 pieces
Microcentrifuge rack	1 piece
15 mL Falcon tube	4 pieces
Cuvette rack	1 piece
Capillary tube	2 pieces
Calculator	1 piece
Scratch paper for calculating	1 Set
Gloves	3 pairs
Tissue papers	5 pieces
Pencil and sharpener	1 piece
Ruler	1 piece
Marker pen	1 piece
Mask	1 piece
Plastic goggle	1 pairs
Waste container	1 piece

Experiment 2. Plant anatomy

Name	Quantity
4 stems of different plant species labelled as SC, SD, SE and SF. Each species include 2 samples	8 pieces
Microscope	1 piece
Lanceolate needle	1 piece
Glass Slide	10 pieces
Glass cover slip	10 pieces
Filter paper	20 pieces
Razor blade	2 pieces
12% bleach solution	20 mL in bottle
3% HCl solution	20 mL in bottle
7.5% carmine solution	20 mL in bottle
1.5% green methyl solution	20 mL in bottle
Distilled water	20 mL in bottle
Timer	1 piece
Marker pen	1 piece
Carrot slice (serves as a cutting board)	1 piece

Experiment 3. Identification of plant species and make the data matrix

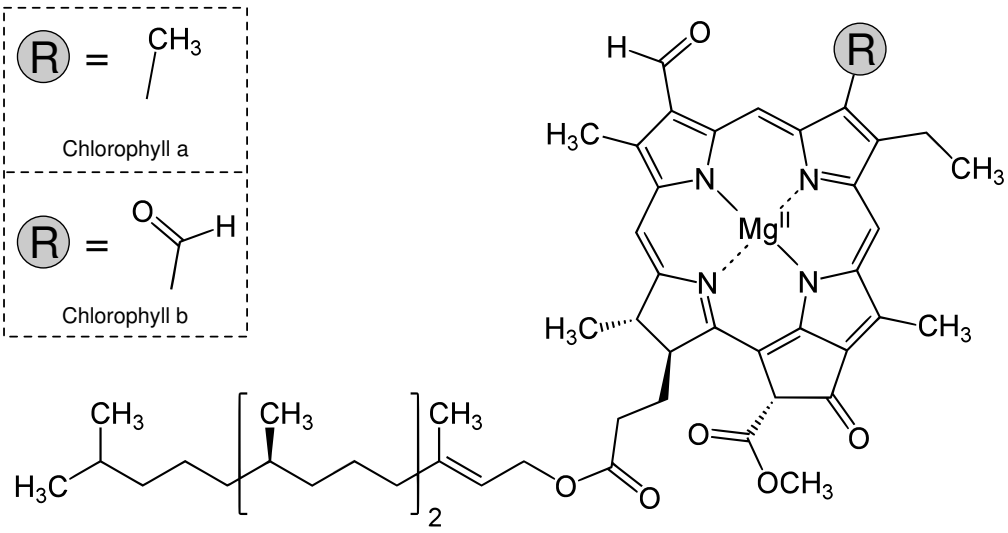
Names	Quantity
5 floral specimen in 70% ethanol labelled as sample PG, PH, PI, PJ, PK. Two flowers for each specimen	5 tubes
Microscope	1 piece
Carrot slice (serves as a cutting board)	1 piece
Magnifier glass	1 piece
Pointed needle	1 piece
Lanceolate needle	1 piece
Glass slide	5 pieces
Cover slip	5 pieces
Forceps	1 piece
Razor blade	2 pieces
Filter paper	5 pieces
Mask	1 piece
Marker pen	1 piece
Distilled water	1 bottle

EXPERIMENT 1. LEAF PIGMENT ASSAY (30 POINTS)

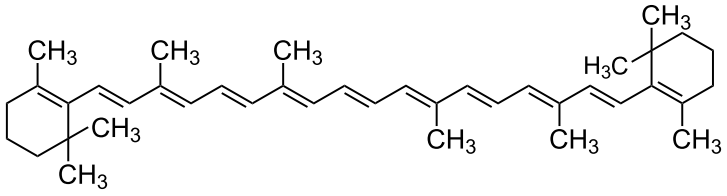
Introduction

Acclimatization to different light intensities involves changes in several physiological characteristics. Leaves exposed directly to sunlight (sun leaves) show differences in leaf structure and their pigment compositions compared with leaves grown under canopy condition (shade leaves). This acclimatization of leaves can be recognized by qualitative and quantitative identification of leaf pigments.

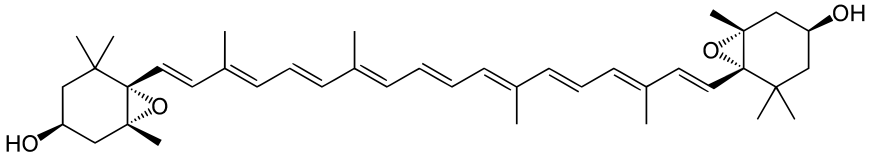
Thin layer chromatography (TLC) is a technique for separating and analyzing the different pigments in the mixture. Leaf pigments including chlorophyll a, chlorophyll b, carotenoids and xanthophylls (violaxanthin, neoxanthin) (see formulas below) can be visualized on TLC plates. To determine the amount of each pigment, leaf extract is quantified by using spectrophotometry at different wavelengths.



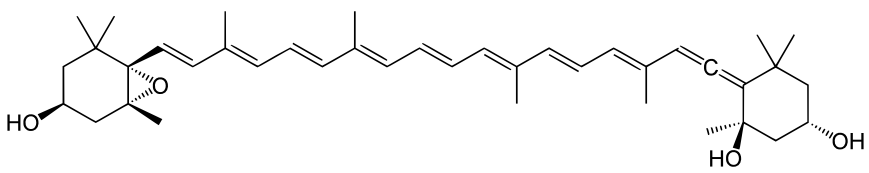
Chlorophyll a and b



b- carotene

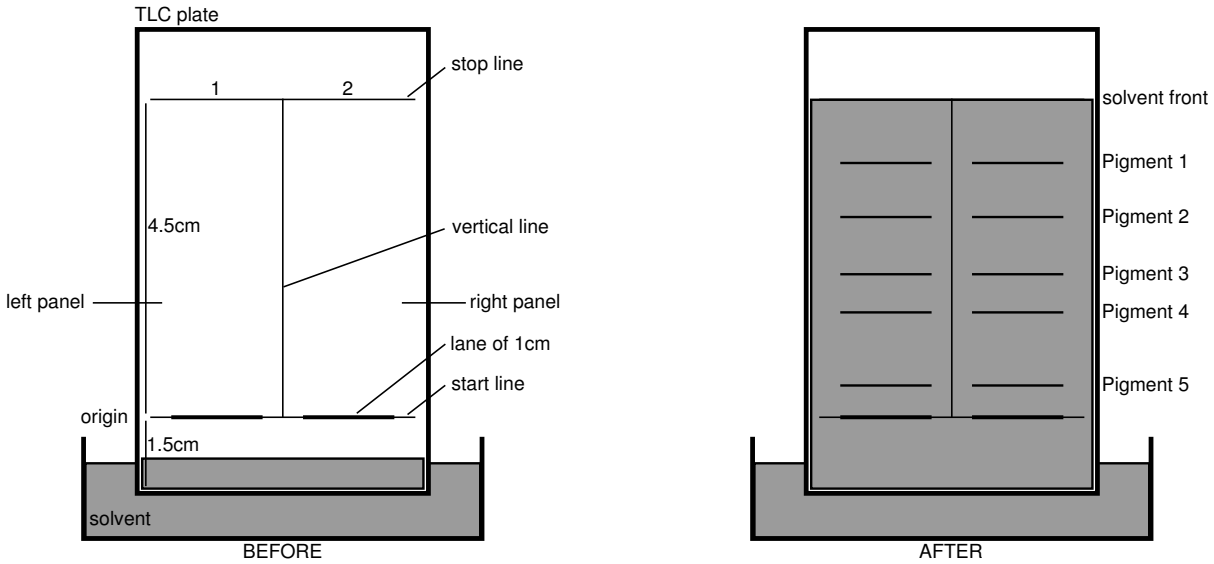


Violaxanthin



Neoxanthin

In this experiment, soybean plants are grown under either sunlight condition or canopy condition to collect leaf samples for qualitative and quantitative analyses of leaf pigments.



Thin layer chromatography of the plant extract

Spectrophotometer

Experiment procedure

1. Grind each leaf sample using a separate mortar and its pestle with 2 mL 95% ethanol into a fine mixture. Add a further 5 mL of 95% ethanol to the mixture, and continuously grind to a homogeneous mixture. Transfer the mixtures onto separate filter papers placed into the funnel. Collect the extract into a labeled 15 mL Falcon tube up to 5 mL resulting in extract A and extract B.
2. Transfer 0.5 mL of each extract into newly labeled 15 mL Falcon tubes.
3. Dilute each extract to 5 mL with 95% ethanol and mix the solution gently.
4. Transfer the diluted extracts A and B to the labeled cuvette A and B, respectively. Measure absorbance at 649 and 664 nm for both cuvette A and B. (*Raise your green card when your cuvettes are ready. The assistants will take your samples to measure and give absorbance values back to you. While waiting for the measurement, you should continue with the next steps of the experiment*).
5. Using a ruler and pencil, lightly draw across the TLC plate 1.5 cm from its bottom edge to make a start line. Place a mark 4.5 cm from the start line to determine the stop line . Lightly draw a vertical line to divide the TLC plate into 2 panels: left panel and right panel. Very lightly draw a 1cm line in the center of the left panel as well as right panel at the start line for indicating loading point.
6. Transfer approximately 0.5 mL of extract A (without dilution) into a 1.5 mL centrifuge tube. Use a glass capillary tube to take extract A from centrifuge tube and load the extract A along the 1 cm line of the left panel. Allow the solvent to dry slightly and apply the pigment again up to 10 times. Similarly load the positive control 10 times onto the lane along start line of right panel of TLC plate.
7. Let the plate dry for approximately 1 minute at room temperature. Put the plate into the TLC bottle containing a shallow pool of chromatography solvent and close the lid (the pigment area on the plate must not be in contact with the chromatography solvent). As the eluent reaches the stop line, remove the TLC plate immediately from the TLC bottle. (*Raise your* **GREEN card***, the assistant will take a photograph of your TLC plate result for grading*). **6 points** will be graded to your photo of TLC plate.

Answer following questions in the <u>Answer sheet</u>

Q.1.1 (10 POINTS)

Record the absorbance values into a table in **Answer sheet**. Calculate concentrations of chlorophyll a (Chl a), chlorophyll b (Chl b), total chlorophyll (Total Chl) according to the following formulae (Lichtenthaler, 1987). Calculate the ratios of chlorophyll a to b for extract A, extract B (to 2 decimal points).

$$\text{Chl a (mg/L)} = -5.19 * (A_{649}) + 13.36 * (A_{664})$$
$$\text{Chl b (mg/L)} = 27.43 * (A_{649}) - 8.12 * (A_{664})$$
$$\text{Total Chl (mg/L)} = 22.24 * (A_{649}) + 5.24 * (A_{664})$$

Q.1.2. (2 POINTS)

Indicate in the **Answer sheet** if each of the following statements is True or False by using this mark ✓

A Leaves used for extract A are generally thicker than those of extract B
B Leaves used for extract A are more sensitive to photoinhibition
C Extract A is derived from shaded leaves
D Leaves for extract A have lower light compensation point than that of leaves for extract B

Q.1.3. (6 POINTS)

After taking the photograph of your TLC plate, place it back into the small plastic bag. Seal the top and staple it to your **Answer sheet.**

Q.1.4. (10 POINTS)

Calculate the Rf values according to the following formula and determine the name of each pigment. The ***distance travelled by pigment*** is measured from the start line to the horizontal and vertical centre of the pigment band. The **distance travelled by solvent** is measured from the start line to the solvent front.

$$Rf = \frac{\text{Distance travelled by pigment}}{\text{Distance travelled by solvent}}$$

*no band = no score

Q.1.5. (2 POINTS)

Indicate in the **Answer sheet** if each of the following statements is True or False by using this mark ✓

A Chlorophyll a and chlorophyll b show different Rf values due to their molecular weight.
B Rf value of chlorophyll and b-carotene are different owing to their different polarity
C Speed of pigment movement mainly depends on interaction with stationary phase on TLC plate.
D In the chromatography experiment, n-hexane and acetone are combined as chromatography solvents. These two solvents are used in order to enhance the solubility of different pigments.

EXPERIMENT 2. PLANT ANATOMY (31 POINTS)

Land plants evolved from algae nearly 500 million years ago. Many new features facilitating survival and reproduction on dry land emerged after land plants diverged from their algal relatives. In land plants, the stem is one of the most important organs that supports leaves and reproductive organs by means of mechanical strengthening and transportation of water, minerals and organic compounds. These functions are carried out by the vascular system, including xylem and phloem, which are present in certain plants. The inner structure (anatomy) of plant stem sections can be observed through microscope.
In this task, you will perform stem sectioning of four plants and observe anatomical traits. Based on characteristics of vascular system, a phylogenetic tree representing the evolutionary trend of vascular system and the relationship between given plant taxa, can be generated.

Experimental procedure

1. Slice samples using a razor blade. Make cross-sections as thin as possible.
2. Transfer sections onto a glass slide. Add drops of bleach solution to fully cover the sections and let stand for 2 minutes. Use filter paper to remove excess bleach solution from the sections.
3. Add drops of HCl solution to fully cover the sections and let stand for 30 seconds. After that, use filter paper to remove excess HCl solution from the sections.
4. Add drops of water to wash the sections. After that, use filter paper to remove water from the sections.
5. Add drops of carmine solution to stain the sections for 3 minutes. Use filter paper to remove carmine solution.
6. Add drops of green methyl solution to fully cover the sections and stain for 30 seconds. After that, use filter paper to remove excess green methyl solution.
7. Add drops of water on the sections, cover the sections with a glass cover slip, use filter paper to remove excessive water, and then observe under a microscope.

Answer these following questions into the Answer Sheet:

Q.2.1. (8.0 POINTS)

Which of these following tissues are present in each plant sample?

Mark ✓ for tissues present and × for tissues absent in observed samples in the table in the **Answer sheet**

Q.2.2. (6.4 POINTS)

Use the plant sections to observe 4 stem anatomical characters and state whether they are absent (0) or present (1). Write 0 or 1 of each character to the data matrix in the **Answer sheet** below. The data of outgroup taxon SH are already given.

A phylogenetic tree (shown in Fig. below) of four experimental plant species and one given species (SH) is generated using parsimony method, based on the above data matrix. The primitive character state (state 0) is hypothesized to be the same as the state in the taxon SH.

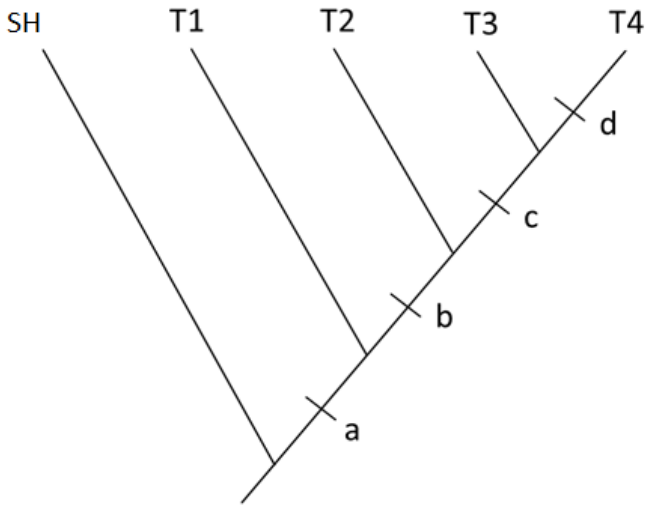


Fig. Phylogenetic tree of 5 species
(Taxon: T)

Q.2.3. (8.0 POINTS)

Using the result from Q.2.2, determine the stem character (C1 to C4) corresponding to the character (a to d) in the phylogenetic tree, and define the position of each experimental plant species (SC to SF) corresponding to its taxon (T1 to T4) in the phylogenetic tree.

- Write down the stem character (C1 to C4) corresponding to the character (a to d) in the phylogenetic tree:

Q.2.4. (6.0 POINTS)

- Write down the name of plant species (SC to SF) corresponding to its taxon (T1 to T4) in the phylogenetic tree:

Q.2.5. (1.0 POINT, 0.25 POINTS EACH)

Refer to the diagram of one vascular bundle on the**Answer Sheet** and label the metaxylem (1), phloem (2), protoxylem (3) and sclerenchyma (4) into the open boxes

Q.2.6. (1.6 POINTS)

Indicate in the **Answer sheet** if each of the following statements is True or False by using this mark ✓

A The stem of plant SC could not transport water as efficiently as plants SD and SE

B The abundance of sclereid in plant SD makes the stem hard.

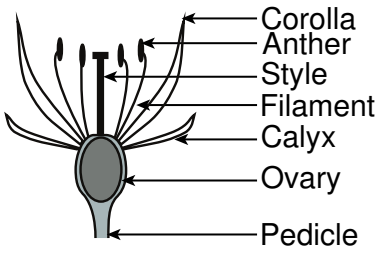
C The stem diameter of plant SE does not increase continuously during plant development because sclerenchyma restricts the development of vascular bundles.

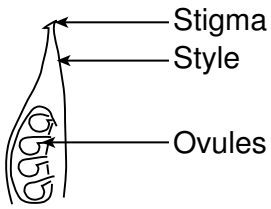
D The sclerenchyma ring below the epidermis strengthens the stem of plant SF.

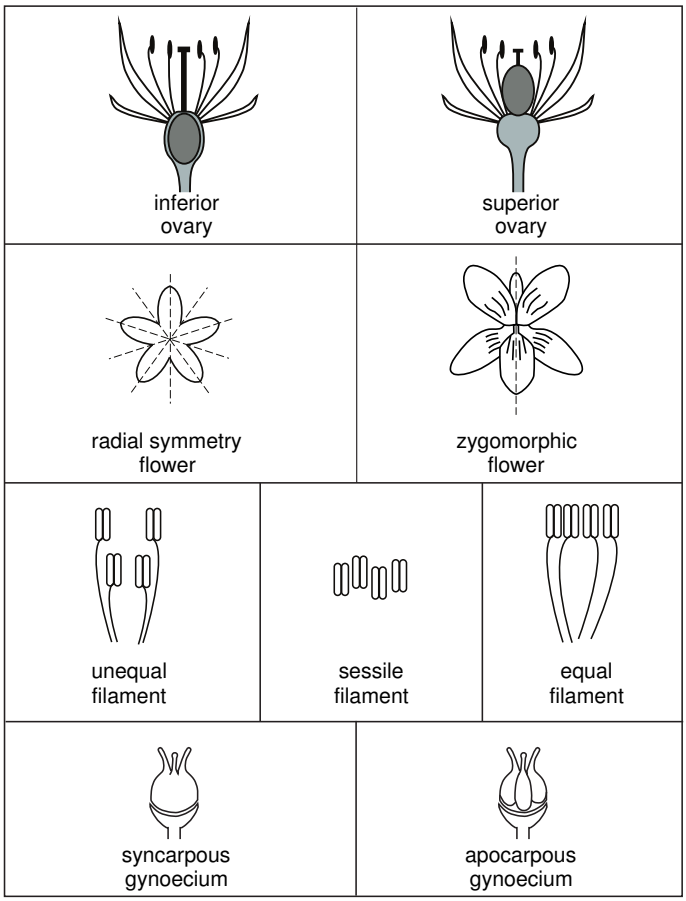
EXPERIMENT 3. IDENTIFICATION OF PLANT SPECIES AND MAKE DATA MATRIX (30POINTS)

Introduction

Flower structures are exceedingly varied but they are useful for plant species identification.
In this task, you will identify morphological and anatomical traits of five given floral samples (sample PG-PK) and answer the questions in the **Answer sheet**. Based on the given dichotomous key and using observed morphological and anatomical characteristics you have to name samples PG-PK. Make data matrix for given samples.
To help understand the terminology used below figures:







Experimental procedure

3.1. Flower symmetry

- Use the forceps to take each sample out of the falcon tube onto a glass slide. Close the tube to avoid ethanol vapor in the room. Use filter paper to remove excess ethanol.
- Handle the flower specimens carefully, as you need the given plant material for all your observations.

Q.3.1. (2.5 POINTS)

Distinguish flower symmetry in each sample and fill "✓" in the table if the flower is radial symmetrical or zygomorphic in the **Answer sheet**

3.2. Number and characteristics of floral parts

- Use the pointed needle, lanceolate needle, razor blade and magnifier glass to analyze in turn the calyx, corolla, androecium and gynoecium for all samples provided.
- Observe all samples carefully and complete Q.3.2 and Q.3.3.

Q.3.2. (4.5 POINTS)

Determine the number of calyx or calyx lobes, corolla lobes, stamens in each sample and put these numbers in table in the **Answer sheet**

Q.3.3. (9.0 POINTS, 0.3 POINTS PER BOX)

Determine the characteristics of calyx, corolla, filament, and gynoecium in each sample and fill "✓" in the table if with characteristic, "x" if without characteristics in the **Answer sheet**.

- Put the gynoecium on the carrot piece, use razor blade to make cross sections of the ovary as thin as possible on one flower and cut along the ovary on the other flower of the same sample PG, PI, PJ (sample PH, PK are already given). Put the sections on the glass slide, add one drop of water on the section, cover with a cover slip. Observe the sections under the microscope.

Q.3.4. (3.0 POINTS)

Determine the number of locules and ovules per locule in each sample and put these numbers in the table in the **Answer sheet**.

3.3. Identify plant species

Dichotomous key to the species: the key was determined by the presence or absence of characteristic. Read the dichotomous key carefully, if you can't find the characteristic in the first line, please move to the second line in the same number.

1	Filaments **equal or sessile**	Go to 2
	Filaments **unequal**	Go to 6
2	Ovary **more than 2**-loculed	Species **ta**
	Ovary **2**-loculed	Go to 3
3	Number of ovule per locule as **1**	Go to 4
	Number of ovule per locule **more than 1**	Go to 5
4	Corolla **with** hair in abaxial	Species **tb**
	Corolla **without** hair in abaxial	Species **tc**
5	Corolla **with** hair in abaxial, **syncarpous** gynoecium	Species **td**
	Corolla **without** hair in abaxial, **apocarpous** gynoecium	Species **te**
6	Corolla **4-5** lobed	Go to 7
	Corolla **more than 5** lobed	Species **tm**
7	Number of ovule per locule only **1**	Go to 8
	Number of ovule per locule **more than 1**	Go to 9
8	Calyx without hair in abaxial	Species **tf**
	Calyx with hair in abaxial	Species **tg**
9	Corolla **with** hair in abaxial; ovary **inferior**	Species **th**
	Corolla **without** hair in abaxial; ovary **superior**	Species **tk**

Q.3.5. (6.0 POINTS)

Using the dichotomous key, identify the name of the species for samples PG-PK, choose and fill the name of species (ta, tb, tc, td, te, tf, tg, th, tk, tm) in the table in the **Answer sheet.**

3.4. MAKE DATA MATRIX

Q.3.6. (5.0 POINTS)

Write down the correct character state of each character to the data matrix in the table in the **Answer sheet.**

End of practical Exam 1