INTERNATIONAL BIOLOGY OLYMPIAD e. V. IBO

Practical Exam "Plant Systematics,
Anatomy & Physiology"

Student Code:

IBO 2018
Tehran, Iran

29th International Biology Olympiad
July 15-22, 2018

Practical Exam

Plant Systematics, Anatomy & Physiology

Total Points: **100**
Duration: **90 minutes**

Please write your student code into the box on the title page.

Use **answer sheet**, which is provided separately to answer all questions.

The answers written in the question paper **will not be evaluated**.

In order to use the flags (the signs on your desk) just put them in the **flag stand** located on the left wall of your desk.

Please ensure that all the materials and equipments are available to you. If anything is missing, put your yellow flag in the flag stand no later that **5 minutes** after beginning of exam. (Any complaints after 5 minutes will not be accepted)

In case of emergencies or questions put your **yellow flag** in the flag stand.

No additional materials will be provided in any case of material loss during experiments.

Please be careful to follow the **safety instructions** while using materials on this task as is noted through protocol.

We suggest you to read the entire protocol before starting the experiments which helps you with time management.

Stop answering and put down your pen **immediately** at the end of exam. Put the entire protocol with the answer sheet in the envelope. Our assistants will collect the envelopes.

Good luck

Write each indicated number in the cell next to it with your own handwriting.

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MATERIALS AND EQUIPMENT (NUMBER OF EACH OBJECT MUST BE NOTICED)

1. Microscope
2. Lab gloves, tissue paper
3. Samplers (100-1000 µL) and blue tips
4. TLC plates (5 plates)
5. Pencil (1)
6. Ruler (1)
7. Tweezers (1)
8. TLC tank with lid (1)
9. Capillary tube (1 box)
10. Transparent tape (1)
11. Safely-taped razor blades (5) **Caution: Do not cut your finger. Take the blades from their yellow-taped site**
12. Band aids (2)
13. Alcohol pad (2)
14. Watch glass (5)
15. Foam (for sectioning)
16. Glass slide and glass slip (5 each)
17. Wash bottle 500 mL (1)
18. Beaker 1000, 50 and 25 mL (1 each)
19. Dropper (1)
20. Pipette filler (1)
21. Pipette 10 mL (1)
22. 1 mL syringe (1)
23. pH- indicator paper
24. Test tube (5)
25. Solution 1 for NO_3 measurement (1) [CAUTION]
26. Solution 2 for NO_3 measurement (1)
27. Color reference strip for NO_3 measurement (1)
28. Solution 1 for $CaCO_3$ measurement (1)
29. Solution 2 for $CaCO_3$ measurement (1)
30- A rack containing:
-One Falcon tube containing TLC solvent (TS: Ethanol: *n*- Propanol; Distilled water, 2:2:1),
- Extracted petal pigments in microtubes labelled as PA to PE,
- Soils washout in falcons labelled as S1-S5
-Leaf extracts in microtubes labelled as L1 to L5
-Sodium hypochlorite solution (cleaning agent)
- Staining solution (Toluidine blue O solution)
- Leaf samples A-E preserved in microtubes
- Microtube containing KOH pellets (1) [CAUTION]
31- Answer sheets (two, 1 for writing the answers and the second one which is labelled as Answer sheet 2 for pasting TLC plates)
32- Green and Yellow flags.

INTRODUCTION

Caryophyllales is the carnation order of dicotyledonous flowering plants. It is a diverse order that includes trees, shrubs, lianas, mangroves, stem or leaf succulents, annuals, and even carnivorous plants. Many members of the order are ecologically specialized to tolerate extreme environments. In the following experiment we are going to investigate ecophysiological features of five members of caryophyllales order in response to different habitats.

Task 1: Betalain and anthocyanin in Caryophyllales

Nearly all flowering plants have coloured petals with red, blue, or purple products of the anthocyanin biosynthetic pathway. Anthocyanin pigments play additional roles in vegetative tissues, providing protection against ultraviolet (UV), herbivores, and pathogens. Moreover, a second group of colourful pigments, the betalains, is found in the plant order Caryophyllales. One of these two types of pigments exists in each family and they are never found together.

The pigments of petals or bracts of five species (plants PA-PE) of Caryophyllales are extracted in microtubes (plants A-E) Each plant belongs to one family. Determine the kind of pigments present in each plant using the protocol 1.

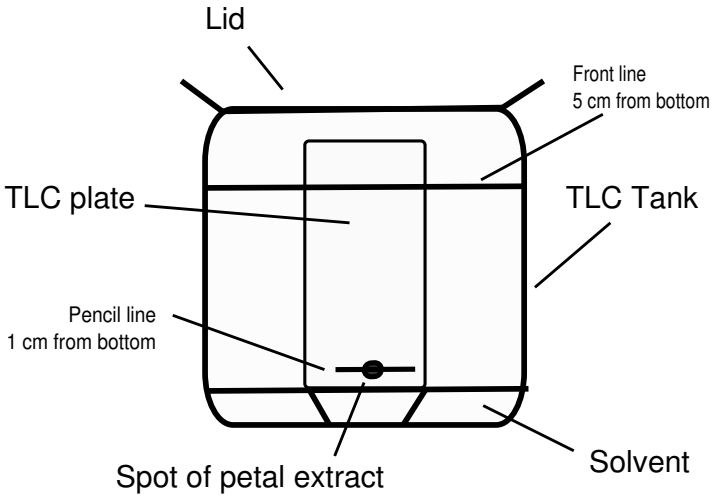
Protocol 1. Anthocyanin and betalain assay:

1.Mark the TLC plates. Draw a straight horizontal line by pencil about 1 cm from the bottom of each plate. (trapez shape. Draw another line 4 cm above the the former one and consider it as the solvent front line).

2. label the TLC plates. Label all the 5 plates from A to E on the top with a pencil (in English alphabet).

3.Take a small amount of solution. Shake the tube before using. Pick an appropriate amount of the pigment extract by using a capillary tube, to spot on the plate.

4.Spotting on plates. As shown in figure 1, gently spot each extract on a separate TLC plate on the horizontal line (Spot radius should be about 0.2 cm). Repeat spotting for each extract to concentrate pigments on TLC plate (about 3 times). It is better to let the TLC plate dry before each spotting.



5. Preparing the tank. Pour the solvent (Falcon TS) to the tank (notice that when you place the plates in the tank, the solvent level should be below the spots). Cover the tank with the lid and wait about **5 minutes** to solvent phase equilibrate with gas phase.

6. Chromatography. When the spots on the TLC plates are dry, gently and immediately (solvent/gas equilibrium must be maintained) place the TLC plates into the solvent tank by tweezers, just in the furrows, with the spotted end of each plate at the bottom.
Note: Put all plates simultaneously in the furrows inside the TLC tank.
Submerge the bottom edge into the solvent. Cover the tank with the lid again. Let the tank be undisturbed. Wait until the solvent reaches the solvent front line.

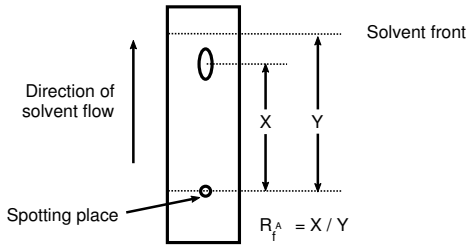
7. Remove the plate. Using tweezers, carefully remove the plate from the tank.

8. Wait for the solvent to evaporate off the plate.

9. Stabilization. Paste transparent tape on your plates to prevent pigment loss and **attach your TLC plates to the answer sheet 2.** Solvent front should be upward.
Note: Once you finished it, put the green flag in the flag stand.

10. Calculate the retardation factor (Rf). Rf is the ratio of the distance a band has moved to the distance the solvent has moved. Use the middle of each band to measure travelled distance. (Figure 2)

Note: Rfs ≤ 0.70 stand for anthocyanin and Rfs > 0.70 for betalain.



Question 1. Indicate presence of anthocyanins or betalians in plants A-E with a "✔" in answer sheet. Also write related Rf-values (Display only two digits of the decimal point and ignore other digits).

To deliver answer sheet 2, put the green flag in the flag stand.

TASK 2: ECOPHYSIOLOGICAL FEATURES OF PLANTS IN ORDER CARYOPHYLLALES

There are five soil washouts indicated as Falcons S1-S5. There are also five leaf extracts (microtubes L1-L5).

Note:The 5 soil washouts (S1-S5) correspond to the 5 leaf extracts (L1-L5), one by one (S1 belongs to L1). But we do not yet know which of these these soil washout and leaf extract pairs belong to which plant (Plants A-E).
By performing protocols 2.1 to 2.3, measure the $CaCO_3$ and Nitrate concentration of soil washout samples and the pH of both soils washouts and plant leaf extracts.

Note: The leaf extracts were prepared at dawn.

Protocol 2.1. Measuring the soil $CaCO_3$ content

To investigate the total concentration of all salts in soil, we measure $CaCO_3$ content of soil washouts by conducting volumetric assay. “Solution 1 for $CaCO_3$ measurement” contains an indicator that turns blue when there is no free Ca^{2+} in the mixture. “Solution 2 for $CaCO_3$ measurement” contains compound X (4 mM) that forms 1:1 complex with Ca^{2+} ions.

1. Add 10 mL of the soil washout to the beaker. (Wash the beaker with distilled water before this step, if necessary).

2. Add 2 drops of the “Solution 1 for $CaCO_3$ measurement” and mix. If the solution is blue, the $CaCO_3$ concentration is ≤1mg/L. If the solution is red, then proceed to next step

3. Using syringe, add “$CaCO_3$ solution 2” to the mixture until the colour turns blue. Shake the beaker while adding the solution. Write the volume of “Solution 2 for $CaCO_3$ measurement”” consumed and Ca^{2+} concentration of soil washouts in **Question 2: Table 1 of the answer sheet** (Ca molar mass = 40 g/mole).

Protocol 2.2: Measuring the Nitrate content

To measure Nitrate content of soil washouts, we use a kit. Instructions of the kit are as follow:

1. Pour 1 mL of the soil washout sample in the 25mL or 50mL beaker.

2. Add 20 drops of the “Solution 1 for NO_3 measurement” c**arefully** and then add distilled water until the total volume reaches 10 mL.

3. **Carefully** and using gloves and tweezers, pick 2 KOH pellets and dissolve them in the above mixture.

4. By adding drops of “Solution 2 for NO_3 measurement”, a yellow colour will appear.

5. Continue to add “Solution 2 for NO_3 measurement” drop-wise and shake until the yellow colour is stable.

6. Transfer about 5 mL of the mixture to a test tube and compare its color with the color reference strip for NO_3 measurement. Record the result in **Question 2: Table 1 of the answer sheet.**

Protocol 2.3: Measuring pH

To measure pH of soil washouts and leaf extracts:

Put one strip of the pH paper into the sample and pull it out after a few seconds. And shake off the paper to remove extra solution.

Determine which colour matches the reference strip and record the corresponding pH in **Question 2: Table 1** in the answer sheet.

According to obtained results answer **Question 2: Table 2 in the answer sheet.**

TASK 3: IDENTIFICATION OF C3, C4 AND CAM PHOTOSYNTHETIC PATHWAY

All three types of photosynthetic mechanisms are known among different families of Caryophyllales. In this task you should determine the photosynthetic pathway of each plant.

You can prepare cross sections with protocol 3.

Notice that **no point** is considered for preparation of cross sections**, but** you may also need your cross sections to answer task 4.

Protocol 3. Preparation of cross sections.

1**. Carefully** prepare free-hand cross-sections from pre-fixed leaves (microtubes A-E) using razor blade and foam pieces. Store the sections in water in the watch glass or on the glass slide.

2. Put hypochlorite (clearing agent) on the sections.

3. Remove the clearing agent after at least **3 minutes**.

4. Wash the sections with water (three times) to get rid of clearing agent.

5. Stain the sections with “Toluidine Blue O” solution. (Dilute the “Toluidine blue O” solution 20 times before using)

6. After **1 minute,** wash the stain away using water.

7. Put the sections on a glass slide. Cover each with a drop of water and then with a glass slip and observe them under light microscope.

8. Based on combined results of task 2 and task 3, indicate the photosynthetic type of each plant with a “✔” **Question 3: Table 1 of the answer sheet.**

TASK 4: CRYSTAL TYPES IN CARYOPHYLLALES

Calcium oxalate crystals are distributed among many members of Caryophyllales. Accumulation of crystals by these plants can be substantial. Major functions of calcium oxalate crystal formation in plants include high-capacity calcium regulation and protection against herbivory. Crystals are formed in specific shapes and sizes. The crystal morphology is species specific and is used traditionally for determination of species and genera in Caryophyllales.

Use the cross-sections of leaves you prepared in the last task in order to identify the crystal type in the plants A-E. The following figure shows the shape of common crystal types.

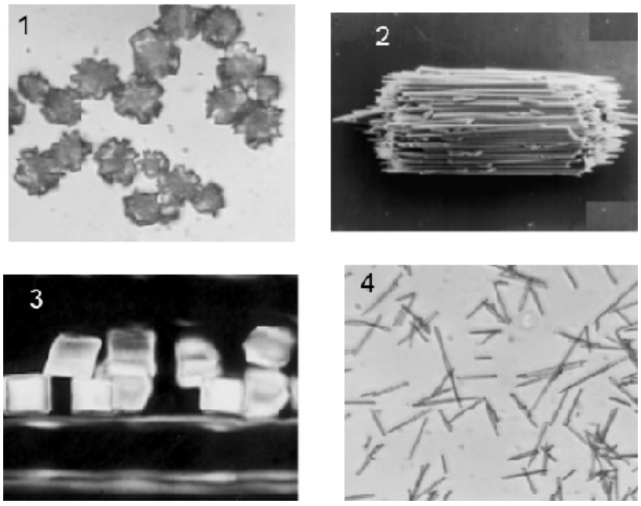


Figure 3. Different types of calcium oxalate crystals, (1) prismatic druse, (2) raphide bundles, (3) tetragonal druse, (4) single raphides.

Question 4:

A: Indicate the presence or absence of each crystal form in plants A-E with with "✔" and "×", respectively. **In the answer sheet.**

B: Given that prismatic druse crystals are abundant in halophytes, soil washout 3 belongs to plant D identify which soil (S1-S5) belongs to which plant (A-E) and fill **the Question 4: Table 2 in the answer sheet.**

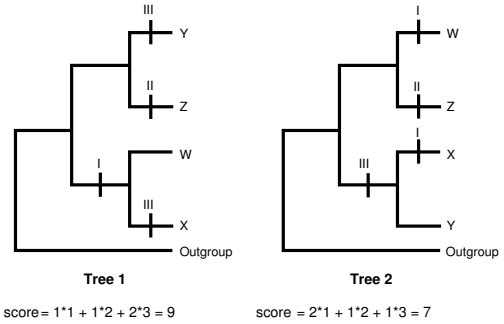
TASK 5: DRAWING A MAXIMUM PARSIMONY TREE

Maximum parsimony is a phylogenetic approach to reconstruct phylogenetic relationships. In this approach, each tree will be evaluated with the number of changes and their weights. The best tree is the one with minimum evolutionary change. Two trees based on the table below for putative taxa W-Z are shown in Figure 4. In this example, 'Tree 2' is better than 'Tree 1' due to its lower score.

taxon \ trait	I	II	III
W	1	0	0
X	1	0	1
Y	0	0	1
Z	0	1	0
weight	1	2	3

$$\text{score} = \sum N_i \cdot w_i$$

N_i is number of changes in trait i in tree and W_i is weight of trait i



The table below shows phylogenetically important traits of putative taxa P-T.

Notice the data in the table does not match with the data you obtained in the previous sections.

Plant \ trait	I (Pigments)	II (druses)	III (raphides)	IV (pollen grain)	V (corolla fusion)	VI (perianth whorls	VII (leaf arrangement
P	0	1	1	1	0	1	0
Q	1	1	0	1	1	0	1
R	0	0	1	1	1	0	1
S	0	0	0	1	0	0	1
T	0	1	1	1	1	1	0
Outgroup	0	0	0	0	0	0	0
Weight	2	2	4	3	2	2	1

The table below lists the states of the traits.

Trait	State 0	State 1
I (pigment)	anthocyanin	betalain
II (druses)	absent	present
III (raphides)	absent	present
IV (pollen grains)	smooth surface	granular surface
V (corolla fusion)	petals free	petals connate
VI (perianth whorls)	1	2 or more
VII (leaf arrangement)	alternate or rosette	opposite

Question 5: Based on the traits in the table above and their weights, draw the maximum parsimonious tree and calculate its score **in the answer sheet**.

Note: Placement of apomorphies on the tree is not necessary and no point is considered for that.