

Country: _____

Student Code: _____

19th INTERNATIONAL BIOLOGY OLYMPIAD

13th – 20th July, 2008

Mumbai, INDIA



PRACTICAL TEST 1

PLANT ANATOMY AND PHYSIOLOGY

Total Points: 47

Duration: 60 minutes

Dear Participants,

- In this test, you have been given the following two tasks:

Task 1: Study of factors affecting the activity of stomata (33 points)

Task 2: Study of plant anatomy and its correlation with the habitat

(14 points)

- **You have to write down your results and answers in the ANSWER SHEET.**

Answers written in the Question Paper will not be evaluated.

- Please make sure that you have received all the materials and equipment listed for each task. In case any of these items is missing, please raise the yellow card.
- At the end of the test, put the Answer Sheet and Question Paper in the envelope. The supervisor will collect this envelope.

Good Luck!!

Country: _____

Country Code: _____

First Name: _____

Middle Name: _____

Family Name: _____

Student Code: _____

Task 1 (33 points)

Study of factors affecting the activity of stomata

You should try and complete this task in 30 minutes.

Materials and equipment	Quantity
1. Specimens labeled 1 to 8 (in red capped vials)	8
2. Compound binocular microscope	1
3. Glass microslides	8
4. Box of coverslips	1
5. Watchglass	1
6. Forceps	1
7. Brush	1
8. Wash bottle containing distilled water	1
9. Permanent marker pen	1
10. Tissue paper roll	1
11. Container for washing and discard	1

Introduction

Stomata are specialized microscopic structures found in all vascular plants.

These microscopic pores allow exchange of gases between the environment and the plant cells. Stomata are also the sites from where water evaporates from the plant.

Various environmental factors such as temperature, humidity and light intensity can affect the opening or closing of the stomata.

Q. 1.1. (3 points) Some statements about stomata are given below. Indicate whether the statements are true or false by putting a tick mark (✓) **in the appropriate boxes in Q. 1.1. in the Answer Sheet.**

	Statement	True	False
a.	Guard cells are the only epidermal cells that contain chloroplasts.		
b.	The Stomatal Index of any plant species is the ratio of number of stomata in a given area of the leaf to the total number of stomata and other epidermal cells in that same area.		
c.	Stomata are characteristic of angiosperms alone.		
d.	Larger the stomatal pore, greater is the rate of transpiration per unit area of the pore.		
e.	The Stomatal Index is always constant for a given species.		
f.	A plant with stomata only on the upper surface of its leaves is most likely to be a submerged hydrophyte.		

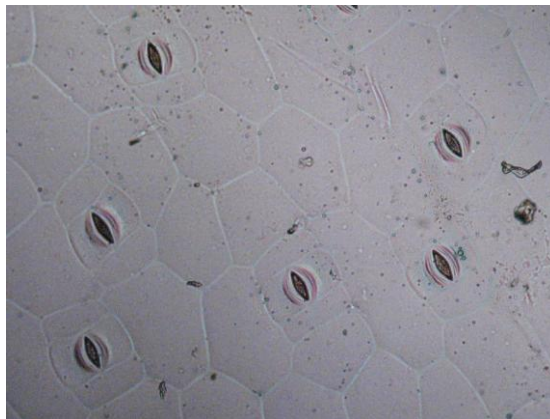
Stomata can be observed by taking an epidermal peel of a leaf. Alternatively, an imprint of the stomata can be obtained, without damaging the leaf tissue, as follows:

A thin coat of transparent colourless nail polish is applied on the leaf surface. The coat is allowed to dry and peeled off using a pair of forceps. This imprint is placed on a microslide with a drop of water, a coverslip is placed on it and it is observed under the microscope.

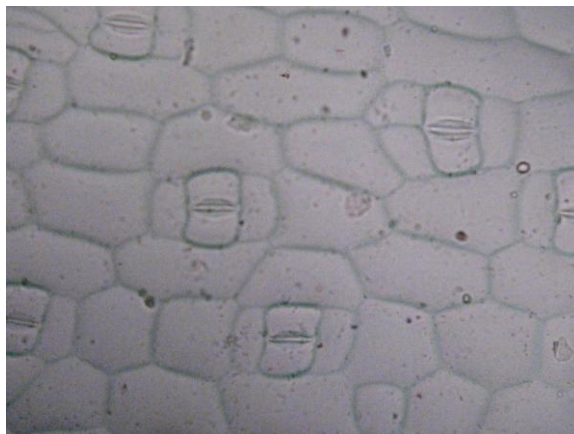
Open and closed stomata can be easily distinguished from these imprints.

Representative microscopic images of the imprints are given below.

OPEN stomata: (10X)



CLOSED stomata: (10X)



In this task, you will study the effect of various factors on the opening and closing of the stomata. These experiments are conducted by immersing leaves in solutions of different chemicals under specified conditions.

Q. 1.2. (0.5 point) The best choice for such an experiment would be:

- a. a mesophyte
- b. a hydrophyte
- c. a xerophyte
- d. a halophyte

Put a tick mark (✓) in the appropriate box **in Q. 1.2. in the Answer Sheet.**

a.	b.	c.	d.

Q. 1.3. (2 points) In one such experiment, a solution of pH 4.0 containing 100 mM K^+ and 0.1 mM Ca^{++} is required. Choose the correct way to prepare this solution from the following options and calculate the amount of KCl and volume of 10mM $CaCl_2$ solution you have to add.

Atomic mass of K is 39.1 and of Cl is 35.5.

- a. Dissolve X g of KCl in 40 ml citrate buffer of pH 4.0, add Y ml of 10 mM $CaCl_2$, and make up the volume to 50 ml.

- b. Dissolve X g of KCl in 40 ml distilled water. Add Y ml of 10 mM CaCl₂ to 5 ml distilled water. Mix both the solutions and adjust the pH to 4.0 with HCl. Make up the volume to 50 ml.
- c. Dissolve X g of KCl in 40 ml distilled water. To this, add Y ml of 10 mM CaCl₂. Make up the volume to 50ml. Adjust the pH to 4.0 with HCl.

Put a tick mark (✓) in the appropriate box and give the correct answer for X and Y in

Q. 1.3. in the Answer Sheet.

a.	b.	c.

X = _____ g KCl

Y = _____ ml 10mM CaCl₂

Experiment

In order to study the effect of various factors on stomata, leaves of a plant were treated in eight different ways. The imprints obtained after each of these treatments (1 to 8 given below) are provided in red-capped vials labeled 1 to 8, respectively.

Please note that these treatments have also been described in the following table for your convenience.

Treatment 1: Leaves were immersed in a solution containing 100 mM KCl, 0.1 mM CaCl₂, pH 7.0 and kept in light for 2 h.

Treatment 2: Leaves were immersed in a solution containing 10 mM KCl, 0.1 mM CaCl₂, pH 7.0 and kept in dark for 2 h.

Treatment 3: Leaves were immersed in a solution containing 0.5 M mannitol, 100 mM KCl, 0.1 mM CaCl₂, pH 7.0 and kept in light for 2 h.

Treatment 4: Leaves were immersed in a solution containing 10 mM KCl, 0.1 mM CaCl₂, pH 4.0 and kept in dark for 2 h.

Treatment 5: Leaves were immersed in solution containing 10 mM KCl, 0.1 mM CaCl₂, pH 7.0 containing an Unknown Chemical and kept in dark for 2 h.

Treatment 6: Leaves were immersed in a solution containing 100 mM KCl, 0.1 mM CaCl₂, pH 4.0 and kept in light for 2 h.

Treatment 7: Leaves were immersed in a solution containing 100 mM KCl, 0.1 mM CaCl₂, 10 µM abscisic acid, pH 7.0 and kept in light for 2 h.

Treatment 8: Leaves were immersed in a solution containing 100 mM KCl, 0.1 mM CaCl₂, 10 µM abscisic acid, pH 4.0 and kept in light for 2 h.

Treatment	KCl	CaCl ₂	pH	Light	Abscisic Acid	Mannitol	Unknown Chemical
1	100mM	0.1mM	7.0	2 h Light	-	-	-
2	10mM	0.1mM	7.0	2 h Dark	-	-	-
3	100mM	0.1mM	7.0	2 h Light	-	0.5 M	-
4	10mM	0.1mM	4.0	2 h Dark	-	-	-
5	10mM	0.1mM	7.0	2 h Dark	-	-	Yes
6	100mM	0.1mM	4.0	2 h Light	-	-	-
7	100mM	0.1mM	7.0	2 h Light	10µM	-	-
8	100mM	0.1mM	4.0	2 h Light	10µM	-	-

Q. 1.4. (8 points): Observation of the imprints

- (i) Pick up the imprint gently from the container using a brush. You may need to gently swirl the vial in order to locate the imprint.
- (ii) Place the imprint in a drop of water on a glass microslide.
- (iii) Place a coverslip and observe under the 10X objective of the microscope. **Note that you will be observing unstained specimens. Hence, make appropriate adjustments in the microscope.**
- (iv) Note down the observations **in Table 1.4. in the Answer Sheet.** You need to count at least 20 stomata per imprint.

Table 1.4.

Treatment	Total number of stomata counted	Number of open stomata	Number of closed stomata
1			
2			
3			
4			
5			
6			
7			
8			

Interpretations

Q. 1.5. (10 points)

Based on the results obtained from the given set of experiments, answer the questions **Q. 1.5.A to Q. 1.5.D in the Answer Sheet**. Fill in the blanks with the appropriate serial numbers from the options given below. Use all options, but each option only once.

- A. The factor/s that clearly lead to stomatal closure is/are: _____
- B. The factor/s that clearly lead to stomatal opening is/are: _____
- C. The factor/s that clearly have no effect on stomatal opening/closing is/are: _____
- D. The factor/s whose effect cannot be clearly established in this experiment is/are:

Options:

1. Light alone
2. Darkness alone
3. Acidic pH
4. Mannitol
5. Unknown Chemical
6. 10 mM KCl alone
7. 100 mM KCl alone
8. CaCl_2
9. Abscisic acid alone
10. Abscisic acid and acidic pH

Q. 1.6. (2.5 points) The correct explanation for the observations in Treatments 7 and 8 is:

- a. Acidification of guard cells leads to opening of K^+ channels of the plasma membrane. This results in entry of K^+ and water molecules to the guard cells.
- b. As the pKa of abscisic acid is close to 5.0, most of the molecules remain undissociated at pH 4.0. This hastens their entry into the guard cells.
- c. No effect was observed in either treatment because there was no water stress.
- d. Abscisic acid is a strong acid and works best at highly acidic pH.

Put a tick mark (\checkmark) in the appropriate box **in Q. 1.6. in the Answer Sheet**

a.	b.	c.	d.

Q. 1.7. (2.5 points) Which of the following correctly explains the effect of mannitol on the stomatal aperture?

- a. Mannitol is a highly hydrophilic substance and restricts the entry of water molecules into the guard cells.
- b. High concentration of mannitol in the extracellular fluid forces K^+ , Cl^- and Ca^{++} to enter the guard cells. This leads to entry of water molecules into the cells as well.
- c. Entry of mannitol into guard cells increases their solute potential leading to uptake of water.

- d. High solute concentration of mannitol results in withdrawal of water from guard cells.
- e. Entry of mannitol in the guard cells is counter-balanced by the efflux of K^+ and Ca^{++} leading to the withdrawal of water from the guard cells.

Put a tick mark (\checkmark) in the appropriate box **in Q. 1.7. in the Answer Sheet**

a.	b.	c.	d.	e.

Q. 1.8. (2.5 points) You have already observed the effect of the Unknown Chemical on stomata (Treatment 5. Leaves were immersed in solution containing 10 mM KCl, 0.1 mM $CaCl_2$, pH 7.0 containing an Unknown Chemical and kept in dark for 2 h.).

These results suggest that the chemical could be useful for:

- a. weed control by increasing the rate of respiration.
- b. keeping plant cuttings fresh over long periods by preventing water loss.
- c. weed control by acting as a wilting toxin.
- d. increasing crop yield in arid lands by increasing rate of photosynthesis.
- e. increasing plant growth by reducing photorespiration.

Put a tick mark (✓) in the appropriate box **in Q. 1.8 in the Answer Sheet.**

a.	b.	c.	d.	e.

Q. 1.9. (2 points) In this task, you studied the effect of various factors on the opening and closing of the stomatal aperture. Similar experiments were performed by scientists and they discovered that light activates zeaxanthin molecules, present in the guard cells, which in turn, activate an ATP-powered proton pump of the guard cell membrane. With this background information and the observations made by you in this task, you have to arrange the sequence of events involved in the response of stomata to light. Fill in the correct options against each step **in Q. 1.9. in the Answer Sheet.**

Mechanism:

Step I: _____ 1 _____

Step II: _____ 2 _____

Step III: _____

Step IV: _____ 6 _____

Step V: _____

Step VI: _____

Step VII: _____

Options:

- 1) Activation of zeaxanthin by light
- 2) Activation of ATP-powered proton pump
- 3) Closing of the stomata
- 4) Influx of K^+
- 5) Efflux of K^+
- 6) Change in membrane potential
- 7) Efflux of Ca^{++}
- 8) Efflux of protons
- 9) Influx of water molecules
- 10) Efflux of water molecules
- 11) Opening of the stomata

Task 2 (14 points)

Study of plant anatomy and its correlation with the habitat

You should try and complete this task in 30 minutes.

Materials and equipment	Quantity
1. Fresh plant specimens	
(i) Leaf in a Petri dish (labeled X)	1
(ii) Stem in a Petri dish (labeled Y)	1
2. Compound binocular microscope	1
3. Razor blades	2
4. Glass microslides	2
5. Box of coverslips	1
6. Watchglasses	3
7. Safranin staining solution (labeled S)	1
8. Brush	1

Introduction

Plants growing in different habitats exhibit various adaptations. These adaptations can be studied macroscopically as well as microscopically and correlated to their habitats.

In this task, you will study the anatomy of the given specimens using the following method. Both specimens X and Y belong to the same plant.

Method

1. Take thin transverse sections of the leaf specimen X.
2. Stain with Safranin staining solution for about 30 to 60 seconds.
3. Wash the section with distilled water and mount on a clean glass microslide in a drop of water.
4. Place a coverslip and observe under 10X objective of the microscope.
5. Repeat Steps 1- 4 for the stem specimen Y.

Observations on the leaf specimen X:

Observe the leaf section and answer **Questions Q. 2.1. and Q. 2.2.**

Q. 2.1. (4 points) Choose the appropriate letters from the Dichotomous Keys 1 and 2 given in **Annexure 2.1.** and fill in **Q. 2.1.I. and Q. 2.1.II. in the Answer Sheet.**

Note: Schematic representations of some of the plant structures are given in **Annexure 2.2.** for your reference.

I. Trichomes

II. Stomata

Q. 2.2. (4 points) Based on your observations on the leaf section, put a tick mark (✓) in the appropriate boxes **in Q. 2.2. in the Answer Sheet.**

	Present	Absent
1. Cuticle	<input type="checkbox"/>	<input type="checkbox"/>
2. Sclerenchyma	<input type="checkbox"/>	<input type="checkbox"/>
3. Collenchyma	<input type="checkbox"/>	<input type="checkbox"/>
4. Aerenchyma	<input type="checkbox"/>	<input type="checkbox"/>
5. Water storage tissue	<input type="checkbox"/>	<input type="checkbox"/>
6. Glands:		
a. Oil gland	<input type="checkbox"/>	<input type="checkbox"/>
b. Salt gland	<input type="checkbox"/>	<input type="checkbox"/>
c. Digestive gland	<input type="checkbox"/>	<input type="checkbox"/>

Observations on the stem specimen Y:

Observe the stem section and put a tick mark (✓) in the appropriate boxes in **Q. 2.3.**
in the Answer Sheet.

Q. 2.3. (3.5 points)

	Present	Absent
1. Cuticle	<input type="checkbox"/>	<input type="checkbox"/>
2. Sclerenchyma	<input type="checkbox"/>	<input type="checkbox"/>
3. Collenchyma	<input type="checkbox"/>	<input type="checkbox"/>
4. Aerenchyma	<input type="checkbox"/>	<input type="checkbox"/>
5. Water storage tissue	<input type="checkbox"/>	<input type="checkbox"/>
6. Vascular bundle:	Open	Closed
	<input type="checkbox"/>	<input type="checkbox"/>
	Collateral	Bicollateral
	<input type="checkbox"/>	<input type="checkbox"/>

Q. 2.4. (2.5 points) Based on your observations of specimens X and Y, identify the type of plant to which they belong.

- a. Mesophyte
- b. Succulent xerophyte
- c. Submerged hydrophyte
- d. Floating hydrophyte
- e. Insectivorous mesophyte
- f. Parasitic mesophyte

g. Halophyte

h. Freshwater hygrophyte

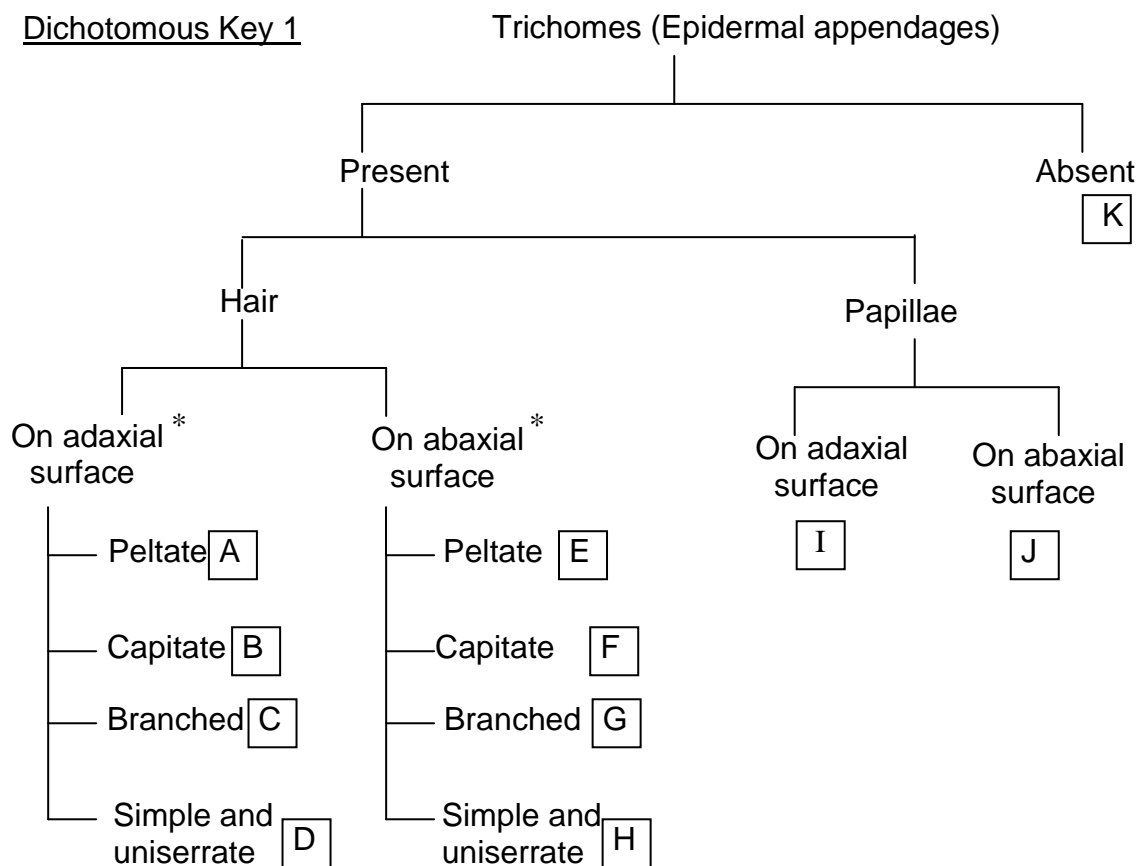
Put a tick mark (√) in the appropriate box in **Q. 2.4. in the Answer Sheet.**

(In this question, the correct interpretation will be given points if it is consistent with your observations.)

a.	b.	c.	d.	e.	f.	g.	h.

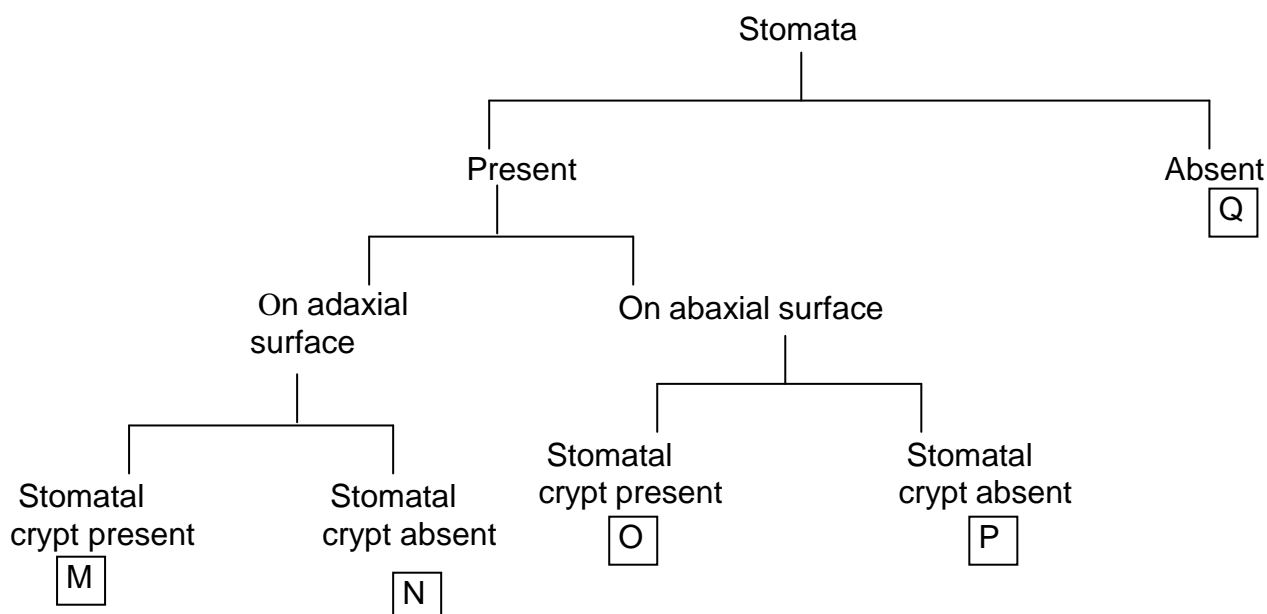
ANNEXURE 2.1

Dichotomous Key 1



* NOTE: Adaxial: facing the stem; abaxial: facing away from the stem

Dichotomous Key 2



ANNEXURE 2.2.

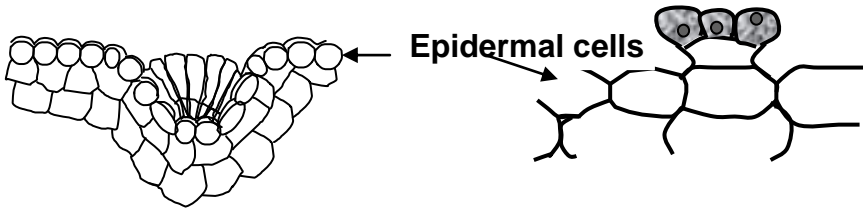


Figure 1: Salt Gland

Figure 2: Digestive Gland

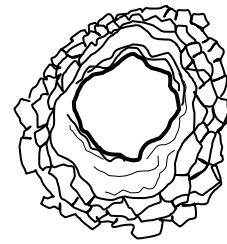


Figure 3: Oil Gland

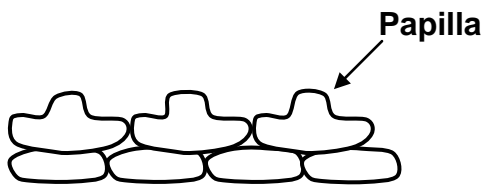


Figure 4: Papillose Epidermis

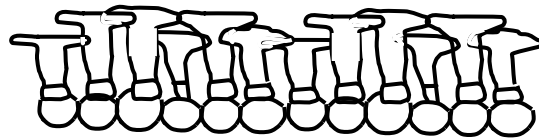


Figure 5: Peltate Hair

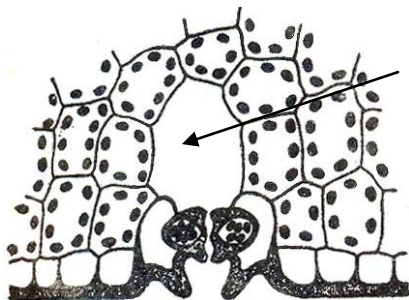


Figure 6: Sunken Stoma

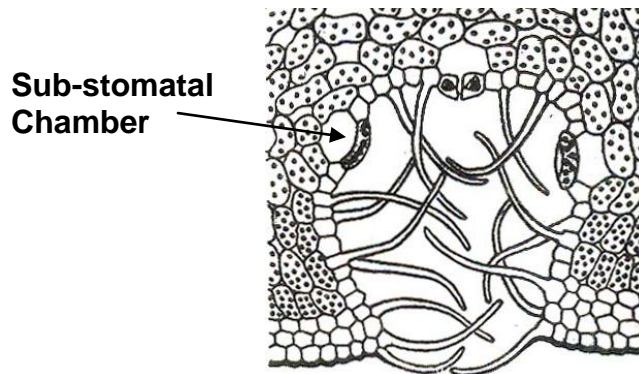


Figure 7: Stomatal Crypt

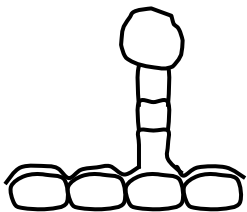


Figure 8: Capitulate Hair

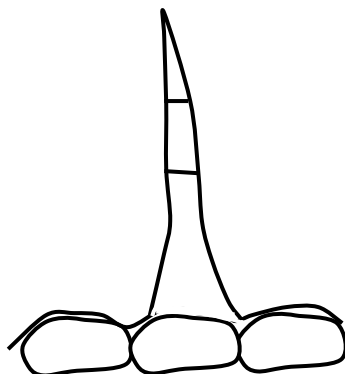


Figure 9: Uniserrate Trichome

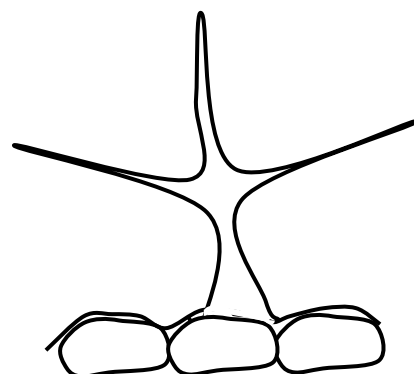


Figure 10: Branched Trichome

*******END OF PRACTICAL TEST 1*******

STUDENT CODE:

Student Code: _____

19th INTERNATIONAL BIOLOGY OLYMPIAD

13th – 20th July, 2008

Mumbai, INDIA



PRACTICAL TEST 1

PLANT ANATOMY AND PHYSIOLOGY

Total Points: 47

Duration: 60 minutes

ANSWER SHEET

Task 1 (33 points)

Study of factors affecting the activity of stomata

Q. 1.1. (3 points)

	True	False
a.		
b.		
c.		
d.		
e.		
f.		

Q. 1. 2. (0.5 points)

a.	b.	c.	d.

Q. 1.3. (2 points)

a.	b.	c.

X = _____ g KCl

Y = _____ ml 10mM CaCl₂

STUDENT CODE:

Q. 1. 4. (8 points):

Table 1.4.

Treatment	Total number of stomata counted	Number of open stomata	Number of closed stomata
1			
2			
3			
4			
5			
6			
7			
8			

Q. 1. 5. (10 points)

- A. _____
- B. _____
- C. _____
- D. _____

Q. 1. 6. (2.5 points)

a.	b.	c.	d.

Q. 1. 7. (2.5 points)

a.	b.	c.	d.	e.

Q. 1. 8. (2.5 points)

a.	b.	c.	d.	e.

Q. 1. 9. (2 points)

Step I: _____ 1 _____

Step II: _____ 2 _____

Step III: _____

Step IV: _____ 6 _____

Step V: _____

Step VI: _____

Step VII: _____

Task 2 (14 points)

Study of plant anatomy and its correlation with the habitat

Q. 2.1. (4 points)

I. Trichomes

II. Stomata

Q. 2.2. (4 points)

	Present	Absent
1. Cuticle	<input type="checkbox"/>	<input type="checkbox"/>
2. Sclerenchyma	<input type="checkbox"/>	<input type="checkbox"/>
3. Collenchyma	<input type="checkbox"/>	<input type="checkbox"/>
4. Aerenchyma	<input type="checkbox"/>	<input type="checkbox"/>
5. Water storage tissue	<input type="checkbox"/>	<input type="checkbox"/>
6. Glands:		
a. Oil gland	<input type="checkbox"/>	<input type="checkbox"/>
b. Salt gland	<input type="checkbox"/>	<input type="checkbox"/>
c. Digestive gland	<input type="checkbox"/>	<input type="checkbox"/>

Q. 2.3. (3.5 points)

	Present	Absent
1. Cuticle	<input type="checkbox"/>	<input type="checkbox"/>
2. Sclerenchyma	<input type="checkbox"/>	<input type="checkbox"/>
3. Collenchyma	<input type="checkbox"/>	<input type="checkbox"/>
4. Aerenchyma	<input type="checkbox"/>	<input type="checkbox"/>
5. Water storage tissue	<input type="checkbox"/>	<input type="checkbox"/>
6. Vascular bundle:	Open	Closed
	<input type="checkbox"/>	<input type="checkbox"/>
	Collateral	Bicollateral
	<input type="checkbox"/>	<input type="checkbox"/>

Q. 2.4. (2.5 points)

a.	b.	c.	d.	e.	f.	g.	h.

***** END OF PRACTICAL TEST 1 *****