INTERNATIONAL BIOLOGY OLYMPIAD e. V. IBO

Plant Anatomy and Physiology Practical

English (Official)

33rd International Biology Olympiad

July 10 - 18, 2022

Yerevan, Armenia

Student Code	

Practical Exam 2

PLANT ANATOMY AND PHYSIOLOGY

Total points: 100

Duration: 90 minutes

Plant Anatomy and Physiology Practical

IBO 33rd 2022 ARMENIA

English (Official)

Dear participants:

This work consists of three tasks.

Task 1. The effect of light on the photosynthetic reactions (35 points)

Task 2. Assessment of plants' vulnerability to predicted climate change (35 points)

Task 3. Species-habitat association (30 points)

GENERAL INSTRUCTION

1. Always wear gloves. If you are working with the scalpel you can temporarily remove your gloves if you want to.

2. Check that you have received all the instruments and materials (see Instruments and accessories). If any are missing, let the lab assistant know by raising the red card during the first 10 minutes of the exam.

3. Use all the materials and instruments appropriately/handle equipment properly.

4. No spilled solution or broken instrument will be replaced.

5. Write your Student Code in the given box on every page of your answer sheet.

6. Record your answers on your answer sheet. Only the answers recorded in the Answer Sheet will be evaluated.

7. Stop writing and put down your pen immediately when you hear the bell ring at the end of the exam.

8. Put all the papers in an empty envelope, and then close it.

9. Wait in your seat until the assistant comes to you to pick up your envelope.

10. No piece of paper, material, stationery, or instrument should be taken out of the lab.

11 colorblind students have the option for invigilators to help compare samples, please raise your red card

WARNING!
YOU MUST ABSOLUTELY ABIDE BY ALL SAFETY RULES AND REGULATIONS INCLUDING WEARING APPROPRIATE PROTECTION, OTHERWISE, THE LAB ASSISTANT WILL ESCORT YOU OUT OF THE LAB.

GUIDELINES FOR FILLING OUT THE ANSWER SHEET

Answers to Plant Anatomy and Physiology Practical assignments are of following types. They are:

1. **numbers** (integer or decimal fraction) in which **the decimal point must be marked with <.> , not <,>**It is allowed to write **a maximum of 3 digits** after the decimal point (for example, whole number: 8; decimal fraction: 0.127), unless specifically requested in the text of the assignment.

2. **an optional answer** indicated by marking the diagonals of the squares with dark red borders (for example, **X**),

Plant Anatomy and Physiology Practical

IBO 33rd 2022 ARMENIA

English (Official)

An **ANSWER SHEET** consisting of 2 pages is provided for filling in the answers to the tasks. The answer forms are processed, checked and evaluated by the computer, that's why it is necessary to follow some rules to fill them:

1. Fill in the answers to the tasks **with a pen** in the answer sheets exclusively in rectangles and squares marked with dark red borders.
2. Write the answers to the tasks clearly and legibly.
3. It is not allowed to make deletions in the answer sheet.

We wish you good luck.

Plant Anatomy and Physiology Practical

Instruments and accessories

- bench lamp
- monocular microscope
- fresh spinach leaf (Spinacia)
- chilled solution labelled 1: “Isolation medium”
- solution labelled 2: “Reaction medium”
- glass beaker wrapped in foil (50 ml)
- mortar and pestle
- stopwatch
- sieve with cheesecloth
- 5 fine glass capillary tubes
- aluminum foil
- red and green filters
- 2 Pasteur pipettes
- ice bath
- table for capillary tubes (in the task set, printed on the paper)
- color scale
- glass rod
- scissors
- marker
- 4 microscope slides
- 4 cover glasses
- 1 tweezers
- 1 scalpel
- 1 razor blade
- 1 inoculation loop, use it as dissecting needle
- 1 small dropper bottle of distilled water (labelled H2O)
- 1 small dropper bottle of HCl 25% solution (labelled HCl)
- 1 small dropper bottle of phloroglucinol solution (labelled 3)
- 1 small dropper bottle of glycerol (labelled 4)
- styrofoam rack with 4 pcs of Eppendorf tubes with plant stem samples labelled II to V
- 3 plants samples labelled B, C, and D in zip-lock bags
- 1 microscope slide of an unknown plant labelled "I"
- 7 photos labelled A, E, F, G, H, I and J
- photo: Fig. 1 Types of stele in vascular plants

- calculator
- filter paper
- paper napkin
- container for used items (labelled Trash)
- gloves
- red card

Clock (stopwatch)

To run the stopwatch:

1. Press M once to enter the stopwatch mode.
2. Press D once to start the stopwatch.
3. Press D once, and the stopwatch stops.
4. Press S once, and the stopwatch resets.
5. Press M once to enter clock mode.

GUIDELINES FOR FILLING OUT THE ANSWER SHEET

Plant Anatomy and Physiology Practical

IBO 33rd 2022 ARMENIA

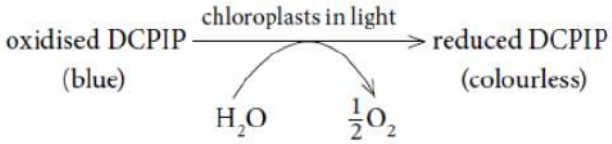
English (Official)

Part 1. The effect of light on the photosynthetic reactions

Assess the effect of light on the light-dependent photosynthetic reactions using dichlorophenolindophenol (DCPIP) as an oxidation indicator.

Introduction

Robert Hill showed that isolated chloroplasts have "reducing power" and liberate oxygen from water in the presence of an oxidizing agent. The "reducing power" was demonstrated by using a redox agent that changed color on reduction. This technique can be used to investigate the effect of light intensity or of light wavelength on the rate of photosynthesis of a chloroplast suspension. Hill originally used Fe^{+3}+ ions as the acceptor, but various other redox agents, such as the blue dye DCPIP can be used instead. DCPIP becomes colorless when reduced:



Procedure

1. Cut the leaves into small pieces and place these in a mortar. Add 5 ml of the chilled solution 1 (isolation medium) and grind the leaves.
2. Quickly filter the extract into a glass beaker covered with foil and put it in the cold water bath.
3. Take a sample by dipping a capillary tube into the extract (tube 1). Put the capillary tube with the sample on the table for capillary tubes and use this as a color standard with which to compare the contents of the subsequent samples.
4. Using a Pasteur pipette, add 2 ml of solution 2 (reaction medium) to the leaf extract and gently mix the contents to start the reaction.
5. With a new capillary tube, take another sample from the leaf extract mixture (tube 2). Quickly lay it on the table for capillary tubes next to the first sample tube and cover this tube in foil to prevent any new exposure to light.
6. Take three more capillary tubes and put them simultaneously in the leaf extract mixture. Record the initial colors of the tubes in the table on the answer sheet in comparison with the color scale.
7. Quickly place one of the tubes on the table for capillary tube under a green filter (tube 3), one under a red filter (tube 4) and one without any filter (tube 5).
8. Direct the light from the lamp on your desk at the tubes and note the color of their contents every 3 minutes for 9 minutes. Record your observations in the table on the answer sheet comparing the colors to the color scale.

Plant Anatomy and Physiology Practical

IBO 33rd 2022 ARMENIA

English (Official)

Question 1.1 (10 points)
Record your observations in a table by comparing and matching the colors of the capillary tube with the color scale. Mark the answer corresponding to the closest matching color with an "X" on the **Answer sheet**. Choose only 1 correct answer for each time point.

		Color of contents every 3 minutes											
Capillary tube №	The content of the capillary tube	0 mins			3 mins			6 mins			9 mins		
1	color standard	K	M	B	K	B	D	M	K	C	V	H	K
2	leaf extract + DCPIP in the dark	K	W	J	X	U	L	W	T	L	X	Q	G
3	leaf extract + DCPIP under the green light	L	W	Y	X	U	L	W	T	L	X	Q	G
4	leaf extract + DCPIP under the red light	X	P	N	R	U	N	R	K	N	B	H	K
5	leaf extract + DCPIP under the white light	X	P	O	V	K	C	V	K	C	B	H	K

Mark the correct answers to questions 1.2-1.6 with an "X" on the **Answer sheet**.

Choose only 1 correct answer

Question 1.2 (5 points)
Which capillary tube is the control?

1	2	3	4	5

Question 1.3 (5 points)
In which capillary tube is the reaction proceeding the fastest?

1	2	3	4	5

Question 1.4 (5 points)
Which 2 tubes will have the same color after 30 minutes from the beginning of the experiment?

№	Capillary tubes	
1	2 and 3	
2	2 and 4	
3	2 and 5	
4	3 and 5	

Question 1.5 (5 points)
Which acceptor does DCPIP substitute?

№	Acceptor	
1	NADP	
2	FAD	
3	O_2	
3	NAD	

Question 1.6 (5 points)
If a blue filter was used, the rate of color change of DCPIC would be between which two tubes?

№	Capillary tubes	
1	3 and 4	
2	2 and 3	
3	1 and 5	
4	4 and 5	

Part 2. Assessment of plant vulnerability to climate change (35 points)

Introduction

Human impact on the environment leads to global warming, which can lead to desertification and the extinction of some biological species.

In the Red Data Book of Armenia, 452 rare species of plants are registered. These species exhibit varying vulnerability to forecasted warming climate conditions, specifically rising temperatures and more frequent droughts. They are divided into 3 groups.

Group 1 includes plant species with relatively wide ecological amplitude and the expected climate change will not be a serious threat to their existence.

For plant species included in group 2, climate change (rising temperatures and more frequent droughts) could be a positive factor, they can even extend their distribution in Armenia.

For plants belonging to the 3rd group, climate change (rising temperatures and more frequent droughts) could be a very serious threat, as it could be difficult for them to acclimate to the altered ecological conditions.

Plants have external and internal structural features, according to which they are divided into 4 ecological groups, which reflect their adaptations to humidity and water availability.

- ***Hydrophytes*** can be submerged or partly submerged, floating or amphibious. In completely submerged plants, stomata are absent. The supporting or mechanical tissues are very poorly developed and the bulk of the tissue is provided with air passages.
- ***Hygrophytes*** are terrestrial plants adapted to the conditions of abundant moisture. Since these plants grow in moist and shady habitats, their rate of transpiration is low, and excess water is expelled through special openings called water stomata or hydathodes.
- ***Mesophytes*** are terrestrial plants found in places with soil and air of moderate humidity. They have thin, large, dark green leaves, covered with a cuticle and thin epidermis. Stomata are unprotected and abundantly arranged on surfaces of the leaves.
- ***Xerophytes*** can grow in dry and extremely dry conditions. Their characteristics include water-storage tissues (fleshy leaves or stems), a decreased leaf surface as a thorn or a scale, may be covered with short, soft hairs or covered in wax. These plants could have deep roots and the stem is often the site of photosynthesis. Some plants that grow on rocks also belong to the xerophytes.

Question 2.1 (5 points)
Identify the ecological groups of the provided plant samples by their morphological and anatomical structures. You are provided with 7 photos labeled **A, E, F, G, H, I** and **J**. In addition, you are also provided with 3 plant samples labeled **B, C** and **D**. Record your observations in a table. Mark the answers to the questions as True (T) or False (F) by putting "X" on the **Answer sheet.**

		A		B		C		D		E		F		G		H		I		J	
№	Ecological groups	T	F	T	F	T	F	T	F	T	F	T	F	T	F	T	F	T	F	T	F
1.0	Hydrophytes																				
2.0	Hygrophytes																				
3.0	Mesophytes																				
4.0	Xerophytes																				

Question 2.2 (15 points)
Identifying stele structures in plants
Introduction
In a sporophyte of vascular plants, the stele is the central part of the root or stem containing the xylem, phloem and in some cases, ground tissue (pith). The species from taxonomic groups are classified according to their stele structure (Figure 1).

Plant Anatomy and Physiology Practical

IBO 33rd 2022 ARMENIA

English (Official)

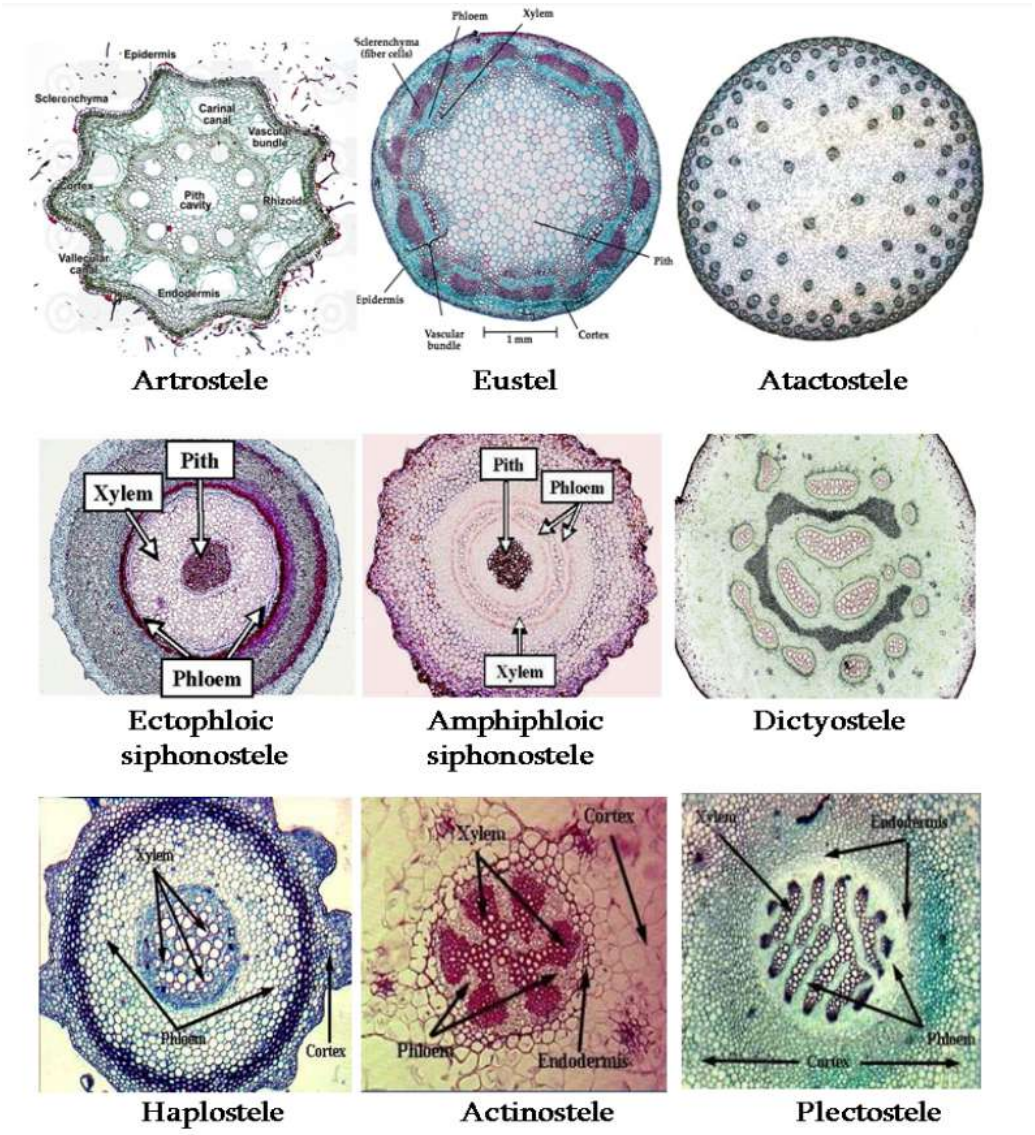


Fig 1. Types of stele in vascular plants

Bryophyta, as non-vascular plants, lack a true stele. Three types of protostele are haplostele, actinostele and plectostele. They are typical for club mosses (Lycophyta). Ectophloic siphonostele is found in fossilized trees. Artrostele is typical for horsetails (Equisetophyta). Amphiphloic siphonostele and dictyostele are typical for ferns (Pterophyta). Gymnosperms and eudicot flowering plants have eustele. Atactostele is typical for monocot flowering plants (Figure 1).

You are provided with one unknown plant microscope slide, labelled "**I**". In addition, you are provided with 4 samples of plant stems, labelled **II, III, IV** and **V**. Prepare cross-sectional anatomical cuts of the 4 plant stems.

Procedure

1. Add a drop of distilled water on a microscope slide.
2. Carefully prepare a very thin cross-section of the stem using a scalpel.

3. Put a slice on the slide and use a inoculation loop to move the stem slice into a drop of water.
4. Conduct Wiesner test for the detection of lignin in xylem transport elements: on top of the drop of water add 2-3 drops of solution 3 (phloroglucinol solution) on the stem slice, remove it after 1-2 minutes with a filter paper and add 1-2 drop of HCl (please be careful with the use of HCL, and wear gloves) solution. Wait for 30 seconds, then remove HCl solution with a filter paper.
5. Add 1-2 drops of solution 4 (glycerol) on the slice and cover it with a cover glass. Wipe a cover glass with a paper napkin if necessary.
6. Observe the stained stem slices under the microscope, and examine the types of steles using Figure 1.

Record your observations in a table. Mark the correct answers to the questions with "X" on the **Answer sheet**. Choose only 1 correct answer for each sample.

		Sample				
№	**Type of stele**	I	II	III	IV	V
1	Protostele					
2	Artrostele					
3	Amphiphloic siphonostele or Dictyostele					
4	Eustele					
5	Atactostele					

Question 2.3 (10 points)
Identify taxonomic groups of the plants. Based on your observations, determine the taxonomic groups of 5 plant samples labeled **I, II, III, IV** and **V**. Record your observations in the table. Mark the correct answers to the questions with "X" on the **Answer sheet**. Choose only 1 correct answer for each sample.

		Sample				
№	**Taxonomic group**	I	II	III	IV	V
1	Lycophyta					
2	Equisetopsida					
3	Pterophyta					
4	Eudicot					
5	Monocot					

Question 2.4 (5 points)
Assess plant vulnerability to expected climate change, specifically rising temperatures.
You are provided with 5 plant stem samples labeled **I** to **V,** which correspond to the photos and plant samples, labeled **A, B, C, D, E** and **F**. Considering the ecological adaptability of species and the taxonomic groups of plants provided, determine whether each group is either resistant or vulnerable. Record your observations in the table. Mark the correct answers to the questions with "X" on the **Answer sheet.**

№	Taxonomic group	Resistant	Vulnerable
1	Lycophyta		
2	Equisetophyta		
3	Pterophyta		
4	Eudicot C4 plants		
5	Monocot C4 plants		

Part 3. Species-habitat association (30 points)

An important aspect of biodiversity conservation is understanding associations between species and their habitats. Soil salinity is an important abiotic stress factor that influences the growth and productivity of plants across the world. In Armenia, semi-deserts that have high-salinity-level soils are the original sites for several important wild ancestors of domesticated wheat varieties.

In this task, you will examine if the species *Triticum araraticum* is associated with the habitat factor of high soil salinity by testing the null hypothesis of no association between the species and the habitat. To do this, you will use the chi-square test, which is conducted using the following formula:

$$\chi^2 = \Sigma \frac{(O-E)^2}{E}$$

where O is the observed and E the expected number of each group.

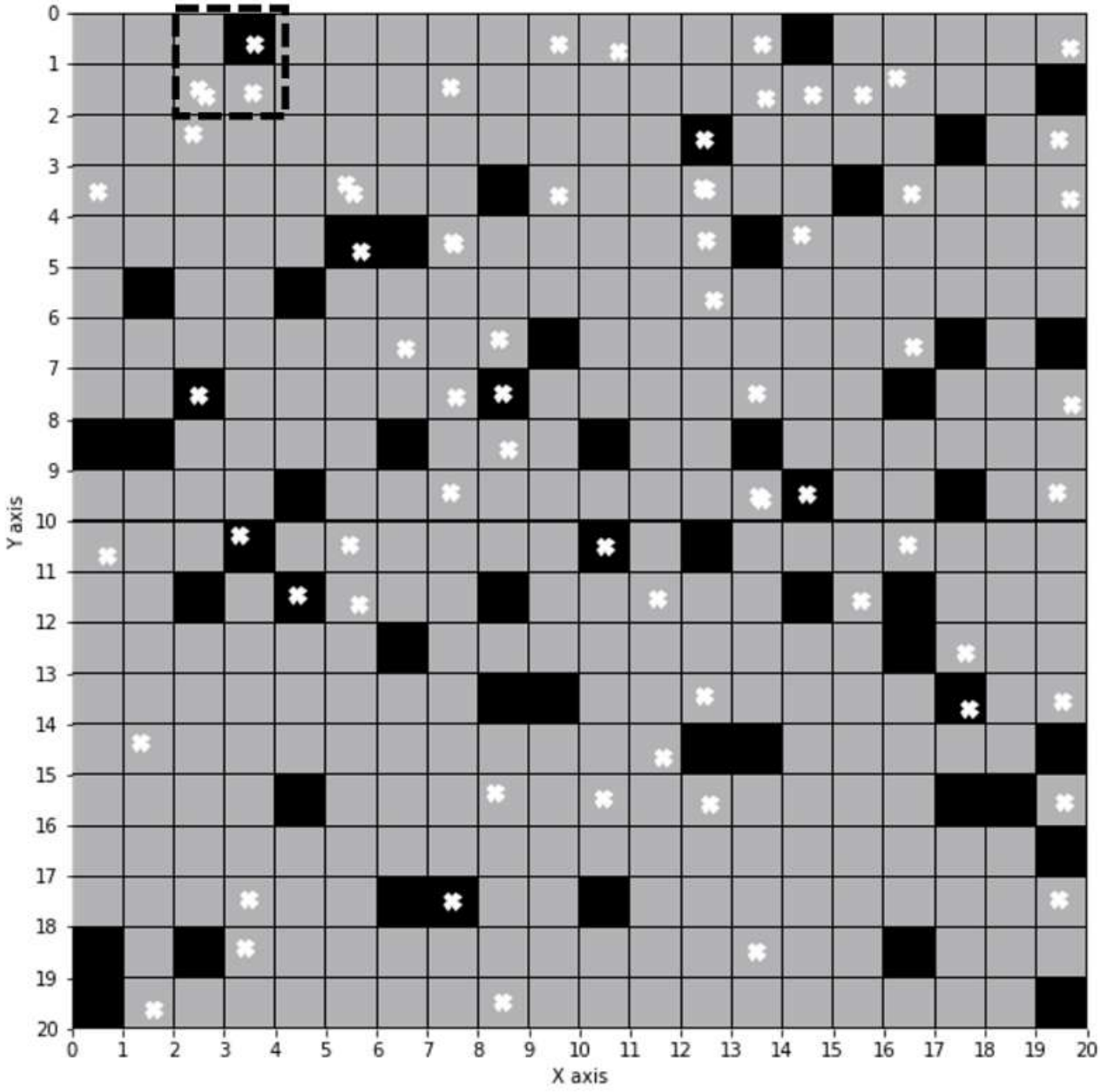
The chi-square statistic is then used to obtain the corresponding p probability from table below (Table 1).

Table 1

	Probability (p)										
DF	0.995	0.975	0.2	0.1	0.05	0.025	0.02	0.01	0.005	0.002	0.001
1	0.0000393	0.000982	1.642	2.706	3.841	5.024	5.412	6.635	7.879	9.55	10.828
2	0.01	0.0506	3.219	4.605	5.991	7.378	7.824	9.21	10.597	12.429	13.816
3	0.0717	0.216	4.642	6.251	7.815	9.348	9.837	11.345	12.838	14.796	16.266
4	0.207	0.484	5.989	7.779	9.488	11.143	11.668	13.277	14.86	16.924	18.467
5	0.412	0.831	7.289	9.236	11.07	12.833	13.388	15.086	16.75	18.907	20.515
6	0.676	1.237	8.558	10.645	12.592	14.449	15.033	16.812	18.548	20.791	22.458

Probability (p) is the probability of observing a test statistic that is as extreme or more extreme than the currently observed test statistic under a statistical model that assumes that the hypothesis being tested is true.

To sample the distribution data of the species and the habitat, you are provided with a grid of species and habitat occurrences (Table 2). In the plot, black squares indicate high salinity, grey squares indicate low salinity. White crosses indicate the presence of *T. araraticum* plant species. An example 4-square quadrat, positioned at the coordinate (3, 1) (the center of the quadrat is at this coordinate), is highlighted with a black dashed square and illustrates a case with both the habitat factor and the plant species present.



Question 3.1 (3 points)
Choose the correct formulation of the null hypothesis. Mark the correct answer with "X" on the **Answer sheet. Choose only 1 correct answer.**

1. There is no significant association between the species *T. araraticum* and spatial soil salinity distribution
2. The distribution of the salinity habitat factor does not affect the distribution of the plant
3. The species and the salinity habitat factor are spatially associated
4. *T. araraticum* is not tolerant to high soil salinity levels

Question 3.2 (9 points)
Fill out the table for each of the random quadrats with the label 1 if the plant or the salinity habitat factor is present (put 1 regardless of the number of occurrences if any are present) and 0 if the plant or the salinity habitat factor is absent. Write the correct answers to the questions in the **Answer sheet.**

N	Center coordinate	High soil salinity	Species *T. araraticum*	N	Center coordinate	High soil salinity	Species *T. araraticum*
1	(7, 14)			16	(12, 4)		
2	(15, 18)			17	(12, 10)		
3	(11, 9)			18	(17, 6)		
4	(8, 2)			19	(10, 10)		
5	(7, 15)			20	(16, 4)		
6	(11, 7)			21	(15, 18)		
7	(11, 12)			22	(15, 12)		
8	(4, 8)			23	(14,15)		
9	(8, 15)			24	(3, 10)		
10	(3, 3)			25	(5, 4)		
11	(2, 14)			26	(7, 14)		
12	(12, 17)			27	(9, 16)		
13	(6, 4)			28	(7, 15)		
14	(2, 18)			29	(18, 8)		
15	(1, 8)			30	(4, 14)		

Question 3.3 (4 points)
Based on these samples, fill out a contingency table for T. araraticum and salinity habitat factor co-occurrence. You need to put the number/count of observations of each category (the number of quadrats where both the plant and the habitat factor are present (1/1), number of quadrats where only plant species is present (0/1) , number of quadrats where only salinity habitat factor is present (1/0), number of quadrats where neither is present (0/0)).

	High soil salinity	
Species *T. araraticum*	Present	Absent
Present		
Absent		

Question 3.4 (2 points)
Based on the contingency table, write down the degree of freedom. Write the correct answer to the question in the **Answer sheet.**

Question 3.5 (3 points)
Calculate E (a) - using the formula below and round up to 1 decimal place (where **a** equals to the number of quadrats where both the plant and the habitat factor are present (1/1), b – only plant species is present (0/1) , c – only salinity habitat factor is present (1/0), d – neither is present (0/0)
Write the correct answers to the questions in the **Answer sheet.**

$$E(a) = \frac{(a+b)\cdot(a+c)}{a+b+c+d}$$

Question 3.6 (4 points)
Based on the values of observed frequencies from Q3.3, your answer from Q3.5 for expected value of category **a** - E(a) and the given values of expected frequencies for the other categories (see table below) calculate the value of the $\chi 2$ statistic and round up to 1 decimal place . Write the correct value in the **Answer sheet.**

	High soil salinity	
T. araraticum	Present	Absent
Present	E(a)	E(b) = 9.6
Absent	E(c) = 5.6	E(d) = 8.4

Question 3.7 (2 points)
Based on your answer and the table of critical values of the chi-square statistic (Table 1), which statement is correct? Mark the correct answer with "X" on the **Answer sheet. Choose only 1 correct answer.**

1. Null hypothesis can be rejected because the probability (p) is smaller than the critical value (0.05)
2. Null hypothesis cannot be rejected because the probability (p) is larger than the critical value (0.05).
3. There is an association between the spatial distribution of T. araraticum and high soil salinity.
4. Assuming the degree of freedom being 2 and the probability (p) threshold being 0.1, the chi-square values smaller than 2.7 are significant.

1		2		3		4	
T	F	T	F	T	F	T	F

Question 3.8 (3 points)
Which statement is correct about the chi-square test of association? Mark the correct answer with "X" on the **Answer sheet. Choose only 1 correct answer.**

1. Negative values of the chi-square test would indicate that *T. araraticum* cannot tolerate high soil salinity .
2. Based on the results of the chi-square test we can conclude whether or not high soil salinity affects the spatial distribution of *T. araraticum.*
3. The greater the difference between the observed and expected ratios, the greater the probability (p).
4. probability (p) should be fixed before calculating the $\chi 2$ statistic.
5. The probability (p) is the probability that the null hypothesis is true.

1		2		3		4		5	
T	F	T	F	T	F	T	F	T	F