

Laboratory work on discipline

"Plant Physiology"

Title Subject: Physiology of plant cells. water metabolism

Lab. Work 1. Plasmolysis. Effect of different anions and cations during plasmolysis.

Purpose: To observe the effect of anion and cation salts on the form and time of plasmolysis.

Assignments:

1. Make a cut from the convex surface of the epidermis of colored onion.
2. Plasmolysis observe under a microscope by various salts.

Guidelines: A slice of the epidermis with the convex surface of the color onion are placed in a drop of the test solution of salt, cover with a coverslip and examined under a microscope. Watch the changing forms of plasmolysis. Determine the time of plasmolysis in each salt.

The results are recorded in the table.

Table

No	Salt	Concentration	Time putting in	Time of beginning of convex plasmolysis	Time of , plasmolysis, min
	1 Ca (NO ₃) ₂	0,7 M			
2	KNO ₃	1 M			
3	KCNS	1 M			

Title Subject: Physiology of plant cells. water metabolism

Lab. Work 2. Cap plasmolysis

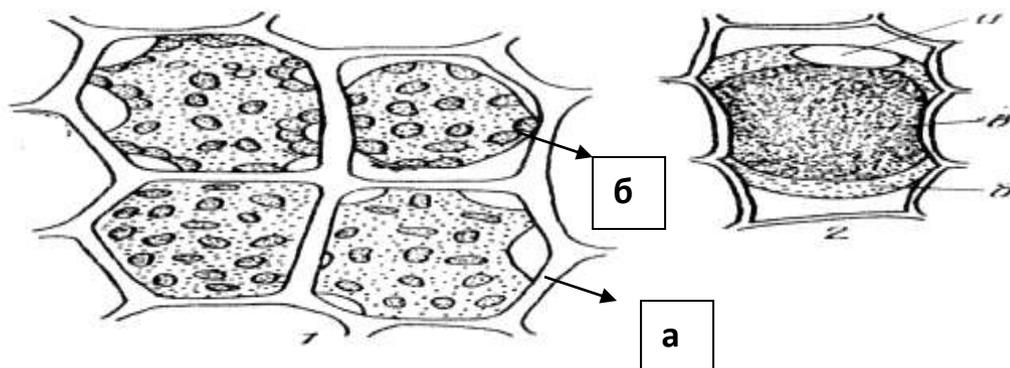
Purpose: Observing the cap plasmolysis

Assignments:

1. Make a cut from the convex surface of the epidermis scales of colored onion.
2. Place the cut in a drop of 1 M solution KSCN. Observe under a microscope.

Guidelines. Make a cut from the convex surface of the epidermis of colored onion, placed on a glass slide in a drop of 1 M solution of KSCN and cover with a cover glass. Sketched a single cell with a distinct cap plasmolysis. Write a conclusion.

Types of plasmolysis



1 - Successive stages of plasmolysis in leaf cells of moss: a - Chevron (угловый) plasmolysis, б - convex (выпуклый) plasmolysis.

2 - cells of the epidermis of onion scales with colored anthocyanin vacuole, -cap plasmolysis: a - nuclear, б – cytoplasm, в - the vacuole.

Title Subject: Physiology of plant cells. water metabolism

Lab. Work 3. Determination of potential osmotic pressure of the cell sap by method of plasmolysis.

Purpose: To determine the potential osmotic pressure of the cell sap by plasmolysis.

Assignments:

1. To prepare the solutions of different concentrations to obtain an isotonic solution.
2. To make a cut from the convex surface of the epidermis scales (чешуи) colored onion.
3. To place the slices in solutions with different salt concentrations.
4. To observe plasmolysis and calculate the potential osmotic pressure.

Guidelines. In preparing beaker 10 ml of 0.7 M, 0.6 M, 0.5 M, 0.4 M, 0.3 M, 0.2 M sucrose solution are prepared by diluting a 1 M solution of dist. water. To make the cut with a convex surface of the epidermis scales colored onion by blade, 2-3 slice are placed in a beaker,. After 30 minutes, the slices were examined under a microscope. Determine the degree of plasmolysis cells in each solution and are isotonic concentration as the arithmetic mean of the concentrations at which plasmolysis just beginning and that at which plasmolysis does not observe.

Write in the table.

Concentration of sucrose, M	On 10 мл solution		Time of duration of tissue in the solutions		degree of plasmolysis	isotonic concentration	potential osmotic pressure of the cell sap
	1M sucrose	H ₂ O, ml	Time of putting	time of observation			
0,7	7	3					
0,6	6	4					
0,5	5	5					
0,4	4	6					

0,3	3	7					
0,2	2	8					

$\Pi = R \times T \times C \times I \times 101,3$, where

R-gas constant: 0.0821 atm/gradhmol; T-absolute temperature ($273^{\circ}\text{S} + \text{room temp}$); C - isotonic concentration in moles; I - isotonic coefficient Vant-Hoff, 101.3 - factor to convert atmospheres in kilopascals.

Write a conclusion.

Lab. Work 4: Identify changes in permeability of the cytoplasm by damaging

Objective: To observe and identify changes in the permeability of the cytoplasm **by damaging**

Assignments:

1. To get the cylinders from red beet root.
2. To place the cylinders in different solutions and one variant has to boil the water along with the cylinder beets.
3. To observe the degree of intensity of staining solution.

Guidelines. From purified red beet root using drill diameter of 0.7-0.8 cm to take 1 cylinders 4 cm. The cuttings cylinders were washed and put one in each of the tubes, in which there are various solutions. One variant is boiled with water. 2 min later cylinder is removed, cooled and immersed in a vial containing 10 ml of cold water. After 30 minutes the tube taken out and compare the amount of pigment released by using photoelektrokolorimeter (FEC) on a blue color filter. The color intensity is expressed by the coefficient of extinction on the scale of the FEC.

Write the data in the table.

Variant	Control (water)	After boiling	Water+6 drops of chlorophorm	30% acetic acid	5% cethanol

Write conclusion on lab. Work.

Title Subject: Physiology of plant cells. water metabolism

Lab. Work 5: Accumulation of dye in vacuoles.

Objective: To observe the accumulation of dyes in vacuoles.

Assignments:

1. Make a cut from the convex surface of the epidermis of onion.
2. Place the slice in a solution of neutral red.
3. Observed under a microscope.

Guidelines. A piece of the epidermis of onion scales kept in a solution of neutral red dye for 20 minutes. Then placed on a piece of colored glass slide in a drop of water. Close the cover glass and examined under a microscope. In living cells vacuoles crimson, and the cytoplasm and nucleus are not colored. Without removing the drug from the glass microscope filter paper with suction water and injected a drop of 1M KNO₃.

To observe changes in the cell used a powerful poison - ammonia. Suck KNO₃. and replace the 10% drop of ammonia. Color becomes yellow - the acid reaction of the cell sap changed to alkaline in the presence of ammonia. Cytoplasm and nucleus are painted in yellow-brown color.

Sketched: 1) the cells of onion, accumulated neutral red in the vacuole, and 2) the dead cells with ammonia.

Write a conclusion based on the obtained results.

Topic: Photosynthesis.

Lab. Work 6: Obtaining of alcoholic extract of green leaf pigments.

Objective: To obtain an alcoholic extract of green leaf pigments.

Assignments::

1. To obtain an alcoholic extract pigments sheet.

Guidelines.

1. 1-2 g of leaves placed in a porcelain mortar, crush, add a little calcium carbonate or quartz sand and pounded. 4-5 ml of ethyl alcohol pour to his mass and then rubbed. After settling, the underside of the spout mortar lightly greased with Vaseline, and the green solution was carefully poured into a glass funnel with a paper filter.

The filtrate was transferred to a volumetric flask of 10 ml, the content of flask adjust to the mark with the solvent, cover the rubber stopper and shake thoroughly.

2. Separation of pigments by Kraus method.

Guidelines. In the test tube pour 2 ml of alcoholic solution of the extract of pigments and add 3-4 ml of gasoline. Contents of the tube is shaken strongly pre-closed hole. Gasoline emulsion layer will turn green because gasoline better solute chlorophyll than alcohol.

Carotene passes in petrol, but paint is masked by chlorophyll. Xanthophyll remains in the alcohol layer, giving a golden yellow color.

Draw the picture of the distribution of individual pigments.

Write a conclusion on lab. Work.

Topic: Photosynthesis

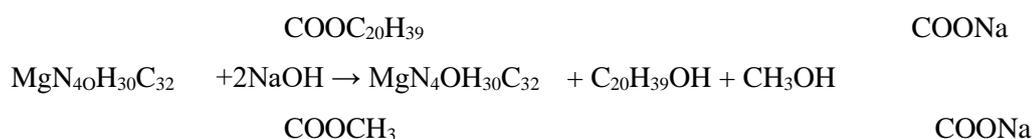
Lab. Work 7. Alkaline saponification of chlorophyll

Purpose: To observe saponification of chlorophyll by adding of alkali.

Assignments:

1. In the test tube with separated by Kraus green leaf pigments add alkali and observe the saponification of chlorophyll by alkali.

Guidelines. In the test tube with pigments separated by Kraus method add dry alkali. Shake and boil. After boiling, the tube cooled. To the cooled solution an equal volume of gasoline and a few drops of water are added. The contents are then shaken and allowed to settle. Carotene and xanthophylls will move in the gasoline layer and sodium salt of chlorophyll acid will move in the alcoholic solution -.



Topic: Photosynthesis.

Lab. Work 8. Photosensitizing effect of chlorophyll on the reaction of hydrogen transfer.

Purpose: To observe the photosensitizing effect of chlorophyll on the reaction of hydrogen transfer.

Task: To Identify the photosensitizing effect of chlorophyll on the reaction of hydrogen transfer using methyl red.

1. Guidelines. Take 4 test tubes:

in the number of 1, 2,3 and 5 ml pour alcohol extraction of chlorophyll,
the 4th - 5 ml of ethanol.

In the number of 1, 2 and 4th tubes add 50 mg crystalline ascorbic acid and solution shaken.

In all tubes with chlorophyll alcoholic solution of methyl red are added br drops until the green color will change to red-brown.

In the 4th test tube color adjusted with methyl blue to bright pink.

Second tube is closed cover of black paper, then all the tubes are illuminated. A water-filled vessel is placed between the tubes and the light source.

After 10-15 minutes of lighting in the 1st test tube methyl red becomes colorless due to recovery and the solution is green. In test tubes color of the solution does not change, because the lack of light, ascorbic acid or chlorophyll methyl red is not restored leucoagent.

Fill the results of lab. work in the table

Variant	The mixture in the test tubes			Conditions of experiment	Results
	Chlorophyl, ml	Ethanol	Saturated alcohol solution of methyl red		

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Draw a conclusion of lab. work.

Topic: Photosynthesis

Lab. Work 9. Determination of the rate of photosynthesis by assimilation flask.

Objective: To determine the rate of photosynthesis by assimilation flask.

Tasks: Identify the rate of photosynthesis by assimilation flask.

Guidelines. Take 2 bottles and wrap their throat pieces of paper.

Stand the flask for 20-30 minutes to fill with air.

Then simultaneously insert tube with holes, closed with glass stoppers.

Cut off a branch, update razor cut under water and place in a water-filled tube attached to a stick inserted into the cork.

Expose to the light bulb.

Duration of the experiment should be such that the leaves do not have time to absorb more than 25% carbon dioxide content.

Fill the results in the table

Sheet No. Ba(OH) 2 Number of HCl, Jr. Amendment titer Int Th f / s.

Objects Объек- ты	Time			Squar e of leaf	Amount of Ba(OH) 2	Amount of HCl, ml		Amendmen t titer Int	Photosynthe- sis intensity
	Star t	En d	Du- ration			experienc e	con- trol		

Write the results as conclusion of lab. work

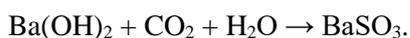
Topic: Plants respiration

Lab. Work 10. Determination of the respiration of seeds in a closed vessel.

Objective: To determine the intensity of the respiration of seeds in a closed vessel.

Tasks: Identify the respiration of seeds in a closed vessel.

Guidelines. The method consists in accounting for the amount of CO₂ released by seed respiration, CO₂ is absorbed by barite.



Excess barite, not react with CO₂ is titrated with oxalic acid.



Guidelines.

4 g of germinated seeds of wheat or rice is placed in a gauze bag. In two conical flasks is filled with 10 ml of 0.1 N Ba(OH)₂ and stoppered.

In one flask, opening quickly append a bag of seeds, add 3 drops of phenolphthalein and titrated with 0.1 N barite oxalic acid to slightly pink color. Titrated as barite in the control flask. In the titration flask stopper through which the tip of the pipette attached to the bottle with barite.

Respiration rate (mg CO₂ allocated 1 g of dry seeds for 1 hour):

$$I = (a-b) : kx2, 2 n,$$

where a and b - the number of 0.1 N oxalic acid at a cost of tirovanie barite, respectively, in the control and experimental flask, ml.

For the correction to the titer.

2.2 - the amount of CO₂ matching 1 ml of 0.1 N oxalic acid,

n - mass of dry seeds

Fill the results in the table

Experimental conditions	Weight of seed g	seed moisture, %	dry weight of seeds, g	Volume barite ml	Amounts of oxalic acid, ml		respiration intensity mg CO ₂ / g dry seeds
					control	experience	

Write the results as conclusion.

Topic: Plants respiration

Lab. Work. 11. Determination of dehydrogenases in plants recovery of methylene blue

Explanations: Dehydrogenase. Enzymes of this group is oxidized substrate by withdrawal of hydrogen. This group of enzymes oxidizes a wide range of substrates. Coenzyme part dehydrogenase represents either pyridine nucleotides (NAD or NADP) or flavin nucleotides (FMN, FAD). Accordingly, different pyridine and flavin dehydrogenase are known.

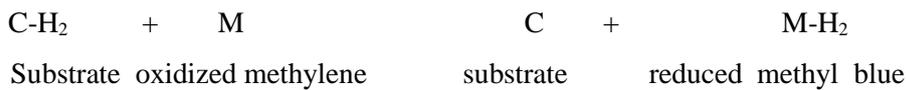
Pyridine dehydrogenase enzymes are anaerobic, they are not able to transfer of reducing equivalents to oxygen. The redox potential of these dehydrogenases is - 0.32 V.

For NAD-dependent dehydrogenases lactate dehydrogenase, malate dehydrogenase, isocitrate dehydrogenase, the NADP-dependent glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase are included

Flavin dehydrogenase may be anaerobic, and aerobic. Oxidation with aerobic flavin dehydrogenase leads to the formation of **hydrogen peroxide**. These dehydrogenases may be called oxidases because of their interaction with oxygen.

Flavin Dehydrogenase have wide variety of oxidation-reduction potential, they often contain metals - copper, molybdenum, and iron.

The activity of the dehydrogenases can be determined as using **methylene blue dye** a hydrogen acceptor, which is recovered becomes a colorless leucoform:



In contact with molecular oxygen leucoform methylene blue is spontaneously oxidized and regains color: $\text{M-H}_2 + 1/2\text{O}_2 \rightarrow \text{M} + \text{H}_2\text{O}$. Therefore, this experiment should be conducted without access to air.

Purpose of lab. work: Determination of dehydrogenases in germinating seeds of peas on the rate of recovery of methylene blue as a function of temperature

Objective germinating seeds of peas or beans, (roots no longer than 0.5 cm).

Reagents: a solution of methylene blue (50 mg/l), distilled water.

Scheme of work:

1. Seeds of peas + methylene blue at room temperature.
2. Seeds of + methylene blue at 30 ° C.

Guidelines.

To peel 10-12 germinating seeds of peas and split them into cotyledons. Half of the plant material placed in a flask with water and boil for 3 minutes to destroy enzymes.

Number the 4 tubes, 1 and 3 put boiled germinating seeds of peas, 2 and 4 normal seeds.

To pour 5 ml of methylene blue in all the tubes with.

After 5-10 minutes, when the cotyledons heavily stained, pour the dye solution.

Thoroughly rinse the plant material with water,

To fill all test tube to the brim with distilled water and close the caps.

Put 1 and 2nd tubes in water bath at 30 ° C.

3 and 4th left in room conditions.

Research results are entered into the table. 1,

Write the results as conclusions in the table.

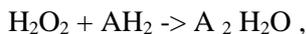
Tab. 1. Effect of temperature on the activity of the dehydrogenases

Objects of study	Experience (incubation temperature)	Intensity of the color of cotyledons
1. Peas	20 °C	
	30 °C	

Topic: Plants respiration

Lab. work 12. Determination of peroxidase in the juice of potatoes

Explanations: Peroxidase - enzymes that oxidize the substrate with hydrogen peroxide. The reaction:



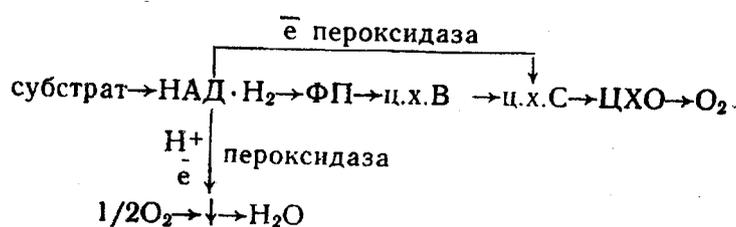
where A and AH₂ - oxidized and reduced substrate, respectively.

Peroxidase substrates are phenols and aromatic compounds, organic hydroperoxide with small aliphatic substituents, NADH (NADPH), naphthohydroquinone, indoleacetic acid, etc.

Peroxidase are - iron enzymes, prosthetic group is a gem - ferriprotoporphyrin IX.

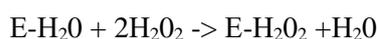
Hydrogen peroxide is formed in the cell as a by-product of the catalytic activity of flavin dehydrogenases in the electron transport chain of respiration. Consequently, the activity of peroxidase disposal of toxic hydrogen peroxide to cells and indirectly associated with the work of the main respiratory ETC.

At the same time it is found that the peroxidase is able to function as a typical oxidase catalyzes the oxidation of the substrate oxygen:



Thus, peroxidase, on the one hand, can oxidize NAD-H₂ by air oxygen, on the other - to transfer electrons derived from NAD-H₂ to the various acceptors (eg, cytochrome c). In both cases, the peroxidase is a link of ETC respiration. Oxidation of substrates is by the one-electron mechanism.

The first step of the catalytic process is the formation of a complex between the iron-enzyme and hydrogen peroxide. Therefore, substrate is oxidated by hydrogen peroxide activated enzyme:



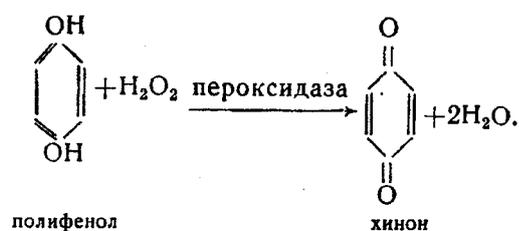
Соединение 1



Compound 1 - oxidized form of the enzyme, where the iron is bound to the peroxide and the valence state of Fe higher Fe³⁺;

compound 2 - product reduction of compound 1 by one-electron reduction by substrate AH₂, AH₂. . substrate reaction, free radical AN-oxidized form of the substrate, and A-product of complete oxidation of the substrate.

Peroxidase determination is based on the formation of colored products in the oxidation of benzidine, guaiacol, hydroquinone, catechol, and other phenols. In this study as an indicator for the detection of peroxidase used hydroquinone. Oxidation of polyphenols to quinones is the scheme:



Objective: to establish the presence of peroxidases in potato tubers, apples.

Objects of study, reagents and equipment.

Objects of research: potato tuber, apple.

Reagents - solutions: 1% hydroquinone, 3% hydrogen peroxide.

Equipment: knife, grater, gauze, funnel, conical cone for 50 ml tubes in racks, pipette 1 and 10 ml.

Study design is shown in Table. 1.

Tab. 1. The effect of high temperature on the activity of the enzyme peroxidase

Variant	The mixture in the test tube			color of the solution in a test tube
	Potato juice (medium peroxidase)	H ₂ O ₂	hydroquinone	
1	+	+	+	
2	-	+	+	
3	+	-	+	
4	+(after boiling)	+	+	

Procedure

Grate some mash potato tuber or peeled apple squeeze out the juice through cheesecloth and collected in a flask. Prepare four tubes and add to them n 5 ml of a 1% solution of hydroquinone.

In a test tube 1 ml of 3% hydrogen peroxide and 1 ml of potato juice are added,

In II tube - 1 ml of 3% hydrogen peroxide solution,

In the III - 1 ml of potato juice

In the IV - 1 ml of potato juice preliminary boiled for one minute and 1 ml of H₂O₂.

In the oxidation of hydroquinone to quinone is browning solution. Some browning of potato juice is observed without the addition of hydroquinone and hydrogen peroxide to it. This is due to the action of polyphenol oxidase, oxidized polyphenols potato tissue with molecular oxygen.

Note the color in the test tube and explain the experimental results, which are recorded in Table. 1.

Based on the data in Table. 1 Write conclusions.

Topic: Mineral nutrition. Microchemical analysis of ash.

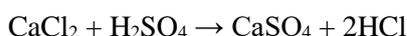
Lab. work 13.

Objective: To carry out microchemical analysis of ash.

Procedure To microchemical analysis of ash.

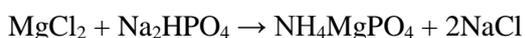
Guidelines. Put into a test tube a small amount of ash and fill it 4-fold volume of 10% HCl. To filter the solution into a clean test tube. Hold on glass reaction of Ca, Mg, P. To do this, the blunt end of a glass rod put on a glass slide a drop of extract and at a distance of 4-5 mm from drop of extract the drop of the appropriate reagent.

Then connect two drops by using arcuate channel. There will be a crystallization of the reaction products. Reagent for Ca is 1% H₂SO₄. This Ca chloride contained in the extract reacts to the acid of the equation.

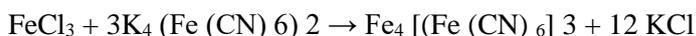


Gypsum is released in the form of needles.

For Mg add a drop of ammonia solution, then combine this solution with the reagent - 1% solution of sodium phosphate. The phospho ammonia magnesium salt. Is formed



For P - 1% solution of ammonium molybdate in nitric acid. The green yellow precipitate of phospho molybdate ammonium salt is formed .



Write a conclusion. Draw the drugs.

Topic: Plants growth and development

Lab. Work 14. Dependence of seed imbibition from the composition of seeds under the absorption of water.

The necessary materials and reagents: the seeds of wheat and peas, technical scales, 2 Petri dishes, filter paper.

In case of contact to moisture seeds increases in volume due to proteins, starch and other hydrophilic colloids absorb water. This process is called imbibition. The reason is that these hydrophilic colloids absorb water are hydrated. Seed storage substances, especially proteins, have high hydrophilic properties. Process of protein hydration consists of three processes:

1) electroneutral hydration - in a protein molecule hydrogen bonds (carboxyl, alcohol, amine, amide, etc. g.). form between polar groups of oxygen and nitrogen/

With such hydration temperature the seed increases along with an increase in. volume of seed,

2) The ion hydration - in a protein molecule ion group - COO⁻ and NH₃⁺ combine with water, pulling the dipole water molecules.

3) Hydration - immobilization of water molecules in the folded protein molecule remnants of free water.

Imbibition activates many biochemical reactions.

The main objective of this work: To prove dependence differences in swelling seeds of different species on the composition of store substances. In the composition of wheat seed protein is 16%, starch - 70%, and in peas seeds of 34% protein, starch - 48%.

Guidelines: In two separate petri dishes 3 g of wheat and peas are placed. Pour water before sinking seeds and leave for 1 hour or day (with decreasing water add water.) Then the water need to pour, the seeds are soaked filtr paper, and then weighed. The ratio of the final value seed weight is expressed to the initial seed weight as a percentage/

Fill the results the table. Plant specie	Seed weight of the plants. gr		Change in seeds weight g	
	Before <u>imbibition</u>	After <u>imbibition</u>	Difference in Weight	The ratio of final weight to initial weight of seeds,%κ
Peas				
Wheat				

Write the results as conclusion and answer on the following questions:

How is the swelling of the seed?

What does the swelling has the composition of seed storage substances?

Topic: Plants growth and development

Lab Work 15. Effect of indolile acetic acid (IAA) on shoots and roots growth of wheat.

Objective: To determine the effect of the IAA on shoots and root growth.

Tasks: To Identify the effect of IAA on shoots and roots growth.

Guidelines. 5 Petri dishes are lined with filter paper, moistened with 9 ml of water or a solution of the following concentrations of IAA: 0, .01, 0.001; 0.0001; 00001%.

To obtain the indicated concentrations of 1 ml of the 0.01% IAA is poured into a volumetric flask of 10 ml, and then is filled with water up to the mark, mixed and placed in Petri dishes. The remainder 1 ml of IAA solution is diluted with water.

On moist filter paper five grains of wheat are laid and closed by lid.

A week later, the length of roots and shoots is measured, conclude about delay of roots and shoots growth or its stimulation depending on the concentration of IAA.

Fill the results in the table.

Variant, concentrations of IAA	length of roots, cm	length of shoots, cm	average length roots on 1 plant	Root length of variant to control,%	Shoot length of variant to control,%

			cm		

Topic. Resistance of plants to extreme factors

Lab Work 15.

Brief explanations: Plants resistance - is their ability to adapt to the adverse effects of the environment, preserving the stability of all physiological processes. The smaller deviation of a process or reaction from the normal as result of exposure to extreme factor and as the sooner they return to normal, so the resistance of plants is higher. Plants mechanisms for sustainability of resistance are different and they can occur at the genetic, the physiological, biochemical and morphological levels.

Lab work. Determination of salt tolerance of cereals at germination of seeds and their growth.

Under conditions of excess soil salinity seeds germination and rate of plant growth is reduced. On defining the level of salt tolerance its parameter is a comparison of the number of germinated seeds and growth at salt solutions and distilled water.

Objective: To determine the salt tolerance of cereals.

Materials and equipment: Petri dishes, filter paper, KMnO₄ or formalin solution (1 ml of formalin per 300 ml of water), beakers, glass cups, labels, a thermostat, pipetting on 10 ml, 7% solution of NaCl, distilled water.

Plants: Seeds of barley, wheat, corn, etc.

Performance of work. Selected healthy seeds put in different glass cups with a label inside and treated with formalin or KMnO₄ solution for 3-5 min for sterilization. Then they are washed by water, lightly dried and. The 10 seeds are laid in each Petri dish. Preliminarily filter paper is stacked on the bottom of petri dishes.

To each Petri dish 10 ml of 7 % aqueous NaCl solution and 10 ml of distilled water (control) are poured. Experiment is carried out in triplicate.

Petri dishes with seeds are placed in the thermostat incubator at a temperature around 26 ° C for germination. After seven days in each variant, the number of germinated seeds is counted. The percentage of germination is determined by comparing it in both experiment variants. In addition, the length of roots and shoots is measured by ruler. The results are recorded in a table (Table 1, 2).

Table. Germination of seeds of cereals, depending on soil salinity

Plant	experiment variants	number of germinated seeds	The percentage of germination %
Wheat	H ₂ O		
	NaCl 7, %		
Barley	H ₂ O		
	NaCl, 7 %		

Table. The length of roots and shoots of cereals, depending on soil salinity

Plant	experiment variants	length of roots	length of shoots	The experiment variants/ control %
Wheat	H ₂ O			
	NaCl 7, %			
Barley	H ₂ O			
	NaCl 7, %			

Fill the results in the table and make conclusion.