PLANT PHYSIOLOGY

Manual for Laboratory Exercises

Jana
AMBROŽIČ-DOLINŠEK

Terezija
CIRINGER

University of Maribor Press

University of Maribor

Faculty of Natural Sciences
and Mathematics

Plant Physiology

Manual for Laboratory Exercises

Authors
Jana Ambrožič-Dolinšek
Terezija Ciringer

June 2026

Title Plant Physiology

Subtitle Manual for Laboratory Exercises

Authors Jana Ambrožič-Dolinšek
(University of Maribor, Faculty of Natural Sciences and Mathematics)

Terezija Ciringer
(University of Maribor, Faculty of Natural Sciences and Mathematics)

Review Zoltán Molnár
(Széchenyi István University)

Andreja Urbanek Krajnc
(University of Maribor, Faculty of Agriculture and Life Sciences)

Translation Jana Ambrožič-Dolinšek
(University of Maribor, Faculty of Natural Sciences and Mathematics)

Language editing Michelle Gay Gadpaille
(University of Maribor, Faculty of Arts)

Technical editors Jan Perša
(University of Maribor, University of Maribor Press)

Alessia Steffè
(University of Maribor, Faculty of Arts)

Graphics material Sources are own unless otherwise noted.
Ambrožič- Dolinšek, Ciringer (authors), 2026.

Cover designer Jan Perša
(University of Maribor, University of Maribor Press)

Cover graphic Photo: Ambrožič-Dolinšek, Ciringer, 2022

Published by
Založnik
University of Maribor
University of Maribor Press
Slomškov trg 15, 2000 Maribor, Slovenia
https://press.um.si, zalozba@um.si

Issued by
Izdajatelj
University of Maribor
Faculty of Natural Sciences and Mathematics
Koroška cesta 160, 2000 Maribor, Slovenia
https://www.fnm.um.si, referat.fnm@um.si

Edition First edition. Translation of the original work from the Slovenian language Ambrožič-Dolinšek, J., Ciringer, T. (2022). Fiziologija rastlin: priročnik z navodili za vaje. Maribor: Univerzitetna založba. doi: 10.18690/um.fnm.3.2022

Published at Maribor, Slovenia, June 2026

Publication type E-book

Available at https://press.um.si/index.php/ump/catalog/book/1127

CIP - Kataložni zapis o publikaciji
Univerzitetna knjižnica Maribor

581.1(075.8)(076.5)(0.034.2)

AMBROŽIČ-Dolinšek, Jana
Plant physiology [Elektronski vir] : manual for laboratory exercises / authors Jana Ambrožič-Dolinšek, Terezija Ciringer ; [translation Jana Ambrožič-Dolinšek]. - 1st ed. - E-knjiga. - Maribor : University of Maribor, University of Maribor Press, 2026

Način dostopa (URL): https://press.um.si/index.php/ump/catalog/book/1127. - Prevod dela: Fiziologija rastlin

ISBN 978-961-299-156-2 (PDF)
doi: 10.18690/um.fnm.3.2026
COBISS.SI-ID 280497411

ISBN 978-961-299-156-2 (pdf)

DOI https://doi.org/10.18690/um.fnm.3.2026

Price Free copy

For publisher Prof. Dr. Zdravko Kačič,
Rector of University of Maribor

Attribution Ambrožič- Dolinšek J., Ciringer T. (2026). *Plant Physiology: Manual for Laboratory Exercises*. University of Maribor, University of Maribor Press. doi.: 10.18690/um.fnm.3.2026

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section 1

PLANT PIGMENTS

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1. Exercise:

Anthocyanins and Betacyanins

INTRODUCTION

Anthocyanins are water-soluble flavonoids in glycoside form (with bound sugars) that accumulate in vacuoles. The aglycones of anthocyanins, which lack sugars, are anthocyanidins.

These pigments are found only in plants, in various tissues such as autumn leaves, flowers, and fruit, that are red, pink, purple, or blue.

The most common anthocyanins are as follows:

- cyanidin (blue, purple, or red anthocyanidins found, for example, in the flowers of the *Centaurea cyanus*, the fruit of rosehip (*Rosa* sp.), blackcurrant (*Ribes nigrum*), and cherry (*Prunus avium*)).
- delphinidin (a blue anthocyanidin found in the flowers and fruit of some plants, such as *Delphinium* sp., *Vaccinium myrtillus*, and blueberry);
- pelargonidin (a red purple anthocyanidin found in the flowers and fruit of many plants, such as bloodworts (Geraniaceae), and berries (*Fragaria* sp.);

- malvidin (blue, found in the petals of *Primula* plants (*Primula × polyantha*), in the blue flowers of blue pimpernel (*Anagallis monelli*), and in red wine from grapevine (*Vitis vinifera*);
- petunidin (a dark red or purple pigment found in many blackberries (*Rubus*), for example in chokeberries, and in red wine, especially from grapes (*Vitis rotundifolia*).

Anthocyanins are involved in interactions between plants and animals. Anthocyanins in the flowers of certain plant species attract pollinators, while those in fruit attract animals that eat them and thus participate in the dispersal of seeds and fruit. In the photosynthetic tissue (assimilatory parenchyma or chlorenchyma) of leaves, anthocyanins protect cells from damage caused by light stress, especially when combined with other stress factors such as cold and drought. Anthocyanins are antioxidants that protect plants from the effects of UV light. They are used as food additives, labelled E 163, to colour jams, fruit bakery products, candies and creams. Their colour depends on their structure (sugar molecule bonding, the number of hydroxyl (-OH), and methoxyl ($-OCH_3$) groups on the B ring of the anthocyanidin), the presence of certain metal ions (Fe, Al, Cr), and the pH of the vacuole.

Betacyanins are red dyes, with nitrogen (N) bonded in the heterocyclic ring. They are found in the vacuole as water-soluble protein complexes. Betacyanins occur in the root of beetroot (*Beta vulgaris*) and other members of the Chenopodiaceae family, as well as in the flowers of the cactus family (Cactaceae), the Portulacaceae family, some species of amaranth (*Amaranthus* sp.) and certain higher fungi. They are used as food additives under E 162 as natural food colouring.

OBJECTIVES

- Learn about the phenolic reaction characteristic of anthocyanins and betacyanins.
- Prepare a pH indicator and measure pH.

TASK

1. Determine the type of red dye in the extract using the phenol reaction ($FeCl_3$).
2. From the anthocyanin extract, prepare a colour scale for the pH indicator.
3. Using the prepared pH indicator, measure the pH of wine, milk, tomato, lemon, and other substances.
4. By changing the pH, observe the colour change in the anthocyanin-stained cell sap (vacuole)

MATERIALS: Red cabbage (*Brassica oleracea* var. capitata rubra), beetroot (*Beta vulgaris* var. conditiva), 2 beakers (500 ml), cooker, test-tube rack, 20 test tubes, FeCl3, alcoholic felt-tip pen.

PROCEDURE

Prepare aqueous extracts of the colouring agents by boiling beetroot and red cabbage pieces separately. Fill one test tube (1−2 cm high) with red cabbage extract and another with beetroot extract.

1. Determine the type of red dye in the beetroot and the red cabbage extract tubes by performing a phenol reaction: add $FeCl_3$ dropwise and observe the colour. A positive phenolic reaction (dark purple-black colour) indicates the presence of anthocyanins; a negative phenolic reaction (reddish-brown colour) indicates the presence of betacyanins (see Table 1).
2. Prepare the anthocyanin pH indicator standard (colour scale) by pouring the anthocyanin extract into eight test tubes (1 cm high). Place these on a rack in a single row. In the first outer tube (tube 1) on the left, add enough acid (1 M HCl) to turn the extract red. In the first tube (tube 8) on the right, add enough base (1 M NaOH) to turn the extract yellow (see Figure 1, Table 2). Then, using a dropper, transfer the contents from the first outer tube to the next inner tube until the colour changes (see Figure 1, Table 2). Continue this process for each successive tube (from left to right toward the centre and from right to left toward the centre). After arranging the colour scale, evaluate it with pH values to match the colours to the corresponding pH changes (see Figure 1, Table 2).
3. Repeat step 2 with the betacyanin extract.
4. Using the anthocyanin pH indicator, estimate the pH of the various solutions (e.g., washing powder solution, vinegar solution, lemon juice solution, liquid soap solution) and record the values (see Table 3). Pour each solution (1−2 cm high) into five test tubes or small beakers and add the anthocyanin extract. Once the colour of the anthocyanin solutions has stabilized, compare it with the anthocyanin pH indicator standard and estimate the pH.
5. Using a microscope, observe the epidermis of red onion. Add base (1 M NaOH) under the coverslip (at the side) and observe the change in colour of the cell sap. Explain this change.

RESULTS AND OBSERVATIONS

Table 1: Type of pigment determined by phenol reaction

Extract	Phenolic reaction (+ positive, - negative)	Type of pigment (anthocyanins, betacyanins)
red cabbage		
beetroot		

Figure 1: Colour scale with defined indicator pH values 1 to 13.

Table 2: pH indicator scale of anthocyanins and betacyanins

Tube	1	2	3	4	5	6	7	8
Add	HCl →			neutral			←	NaOH
Colour of cabbage extract				blue-violet				
pH				7				
Colour of beetroot extract								
pH								

Table 3: pH measurement of different solutions

	pH value
soil	
soap	
washing powder	
vinegar	
lemon	

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2. Exercise:

Photosynthetically Active Pigments

INTRODUCTION

Light absorption by photosynthetic pigments enables photosynthesis in plants, algae and blue-green algae. Photosynthetic pigments differ in their chemical structure and light absorption properties and include: chlorophylls, carotenoids, and phycobilins.

There are primary and accessory photosynthetic pigments. Of all the pigments involved in photosynthesis, chlorophyll plays the fundamental role. The other photosynthetic pigments are known as accessory pigments. Each pigment absorbs only certain wavelengths of light. The main pigment, chlorophyll a (bacteriochlorophyll in bacteria), absorbs blue and short-wavelength red light and converts it into excitation energy. The accessory pigments absorb light and transfer the excitation energy resonantly to the main pigment, whereas only the reaction-center pigment can undergo charge separation and release an electron. When a photon of light excites a photosynthetic pigment molecule, light of a specific wavelength is absorbed, while the remaining light is either reflected or transmitted by the leaf.

QUESTIONS

Explain the characteristics of different types of plastids: proplastids, chloroplasts, chromoplasts, leucoplasts, amyloplasts and etioplasts.

Describe the structure and function of chloroplasts.

Which pigments are photosynthetically active pigments?

Describe photosystem I and II.

What is fluorescence?

Review your knowledge of the light and carbon reactions of photosynthesis.

What are the anatomical and physiological differences between C4, C3, and CAM types of photosynthetic metabolism?

Chromatography is a laboratory method used to separate different substances in a sample based on differences in the rates at which they move through a stationary phase. The components of the sample pass through the stationary phase with the help of a mobile phase.

Separation occurs because of differences in the physicochemical properties of the sample's constituents, such as size, shape, charge, volatility, solubility, and adsorption. Chromatography is a separation technique that can distinguish chemically similar substances. After separation, a chromatogram is obtained. To qualitatively and quantitatively identify the individual components of a sample, an appropriate detection system is also necessary. A chromatographic system consists of several components: the stationary phase, the chromatographic carrier, and the mobile phase.

There are many types of chromatography, including paper chromatography, thin-layer chromatography, column chromatography, high-performance liquid chromatography (HPLC), and gas chromatography (GC).

Paper chromatography:

The distance that the sample (pigments) travels along the chromatography paper depends on two factors:

(a) the solubility (polarity or nonpolarity) of the dye in each of the chemicals making up the chromatographic solvent (mobile phase). A more soluble, nonpolar pigment will travel farther than a less soluble pigment.
(b) the adsorption of the pigment to the chromatography paper (stationary phase). A pigment that is more strongly adsorbed will move more slowly.

The movement of the components (pigments) in the sample is measured relative to the movement of the solvent and is expressed as relative frontal mobility (Rf). The Rf is calculated using equation (1):

$$\boldsymbol{Rf} = \frac{\boldsymbol{sample\ distance}}{\boldsymbol{solvent\ distance}} \quad (1)$$

OBJECTIVES

- Learn about the different pigments involved in light absorption.
- Learn about the extraction and separation of pigments.
- Learn about the chromatographic separation of substances.
- Learn about the absorption properties of pigments.
- Learn about spectrophotometry.

TASK

1. Separate the pigments in chloroplasts using paper chromatography.
2. Calculate the relative frontal mobility (Rf) for each pigment (see Table 4).
3. Place a test tube containing the extract of the unseparated pigment in front of a light source (flashlight) and observe the colour of the illuminated contents.
4. Measure the absorbance of each separated pigment, the control solvent solution, and the pigment extract in the wavelength range of 350 nm to 750 nm using a spectrophotometer.

MATERIALS

Grass (Poaceae) or leaves of any plant, scissors, cutting mat, pestle and mortar, 100 ml beaker, small beaker, chromatography paper, chromatography chamber with cork stopper, capillary, tube rack, 7 tubes, 7 tube stoppers, light source, spectrophotometer.

CHEMICALS

Acetone, petroleum ether, acetone: petroleum ether (v/v = 1:6), silica sand.

PROCEDURE

1. Cut the chromatography paper to fit the height and width of the chromatography chamber. The paper strip should be slightly narrower than the chamber diameter, so it does not touch the wall of the chamber, and long enough to reach or be immersed about 2 mm in the developing liquid. Draw a starting line for sample application with a pencil 1.5 cm from the lower edge of the chromatography paper.
2. Cut the grass into small pieces with scissors, add a small amount of acetone, and grind the grass thoroughly over an ice bath in a mortar, adding a spatula tip of quartz sand. Decant the dark green coarse acetone extract of the pigments into a beaker placed over the ice bath. Using a capillary (pipette tip), apply the dye extract to the drawn starting line (1.5 cm from the lower edge). Repeat the application several times, allowing the line to dry after each application, until a dark green line is obtained. Store the remaining pigment extract in a tube.
3. Attach the chromatography paper with the applied extract to the stopper and place it in the centre of the chamber so that it is immersed 2−3 mm in the mobile phase (acetone: petroleum ether, v/v = 1: 6). The chromatography paper should not touch the chamber wall. When the mobile phase reaches the final line, remove the chromatogram from the chamber, air-dry it, and mark the solvent front, and the pigment front.
4. Cut out the individual pigment spots from the chromatogram and place each spot separately in a test tube. Cover each spot with acetone to extract the pigment.
5. Measure the absorbance (A) of the extracts from individual-coloured spots of the separated dyes with a spectrophotometer at wavelength from 350 nm to 750 nm and graph the absorbance spectra.

RESULTS AND OBSERVATIONS

Table 4: Individual pigments of extract samples and their Rf values on the chromatogram

Sample	Colour of the spot	Rf	Pigment
F			
E			
D			
C			
B			
A			

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3. Exercise:

Separation of Green and Yellow Pigments from Chloroplasts

INTRODUCTION

See: previous tutorials Plant pigments

OBJECTIVES

- Learn the extraction method.
- Learn how to separate green and yellow assimilation pigments on the basis of solubility.

TASK

1. Separate the pigment extracted into ethanol using two different solvents: petroleum ether and ethanol.

MATERIALS

Grass, 100 ml and 20 ml beakers, cooker, mortar and pestle, test tube.

CHEMICALS

Ethanol, petroleum ether, deionized water.

PROCEDURE

Heat the cut grass leaves in ethanol until boiling. Decant the extract into a beaker. Pour 1−2 cm of the extract into a test tube and add an equal volume of petroleum ether. Add a few drops of water, shake, and allow the mixture to separate between the two solvents. Repeat the process until the dyes are separated.

RESULTS AND OBSERVATIONS

Take photos of the separate pigments.

QUESTIONS

What is achieved by adding water?

How is each phase coloured and which pigment does it contain?

In which solvent are the chlorophylls soluble, and in which are the carotenoids soluble?

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4. Exercise:

Determination of Pigment Based on Solubility

INTRODUCTION

See previous exercises on plant pigments.

OBJECTIVES

- Learn about the chemical properties of certain pigments and the use of appropriate solvents for their extraction.
- Identify the type of dye in red pepper (*Capsicum*).
- Identify the type of dye in hibiscus (*Hibiscus*).

TASK

1. Extract the dyes in red pepper and hibiscus using different solvents, water and edible oil.

MATERIALS

Red pepper powder, dried hibiscus (for tea), two tall 100 ml beakers, glass rod.

CHEMICALS

Water, white edible oil.

PROCEDURE

Pour oil to a height of 2 cm, then add an equal volume of water into two identical tall beakers. Add a small teaspoon of red pepper powder to the first beaker and a small teaspoon of hibiscus to the second. Place a glass stirring rod in each beaker. Observe which solvent extracts the colour from the red pepper and which from the hibiscus.

RESULTS AND OBSERVATIONS

Explain which type of dye is present in the red pepper and which in the hibiscus, and on what basis you determined the type of dye.

section 2

GAS EXCHANGE

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5. Exercise:

Plant Respiration

INTRODUCTION

Seed Respiration

In dormant seeds, mitochondria are still undeveloped. Metabolism and gas exchange are extremely low because the water content is low (<10%). During seed swelling (imbibition), respiration increases significantly and can be measured by simple methods. Oxygen consumption rises sharply, and enzymes stored in the seed are activated. Primary nutrient reserves, mostly simple sugars in the embryo, are used. Oxygen stored in various seed tissues is also consumed during these processes. Mitochondria develop, their number increases, and the amount of their enzymes rises. In some plant species, after a few hours, oxygen consumption begins to stagnate or slightly decreases, usually at the end of imbibition. This is likely due to the poor oxygen permeability of the seed coat (the testa), which is even lower when imbibed. Oxygen passing through the seed coat is also intercepted by phenolic substances, which are typically present in high concentrations. At this stage, metabolism is partly anaerobic, but the seed still undergoes intense enzyme synthesis.

A renewed increase in oxygen consumption is observed when visible signs of germination appear, such as the emergence of the radicle. The embryo intensively uses reserves from the secondary endosperm or cotyledons, and rarely from the hypocotyl. In light, a positive

phototropic reaction can be observed in the germinated seedling. The photosynthetic apparatus also develops, and the germarium switches to an autotrophic lifestyle (Likar and Vogel-Mikuš, 2011). Learn more about plant respiration in the lectures.

Plant respiration can be monitored by determining what is produced and what is used. In respiration, we measure the CO_2 released and the O_2 consumed. This process can be monitored using a Vernier meter to measure the concentration of gaseous CO_2 and O_2.

OBJECTIVES

- Demonstrate that CO_2 is produced during respiration.
- Demonstrate that O_2 is used in respiration.
- Measure the amount of O_2 consumed and CO_2 produced in dry and imbibed seeds.
- Learn about the effect of different treatments (various NaCl concentrations) on seed respiration during germination (3–4 days).

TASK

1. Measure the amount of O_2 consumed and CO_2 produced by dry and imbibed seeds.
2. Measure the amount of O_2 consumed and CO_2 produced by seeds treated with 0%, 1%, 5% and 10% NaCl solutions, for each treatment separately.
3. Compare the measurements for each treatment.

MATERIALS

Three-to-four-day old germinating wheat (*Triticum*) seeds, or seeds of other plants treated differently for germination, with 0%, 1%, 5% and 10% NaCl solutions.
Vernier CO_2 and O_2 gas meter, interface, computer, LabPro software, balance.

CHEMICALS

NaCl.

PROCEDURE

The concentrations of oxygen and carbon dioxide gas were measured using a Vernier CO_2 and O_2 gas concentration meter connected to the Vernier interface. You will monitor the results using LabPro software (Figure 2).

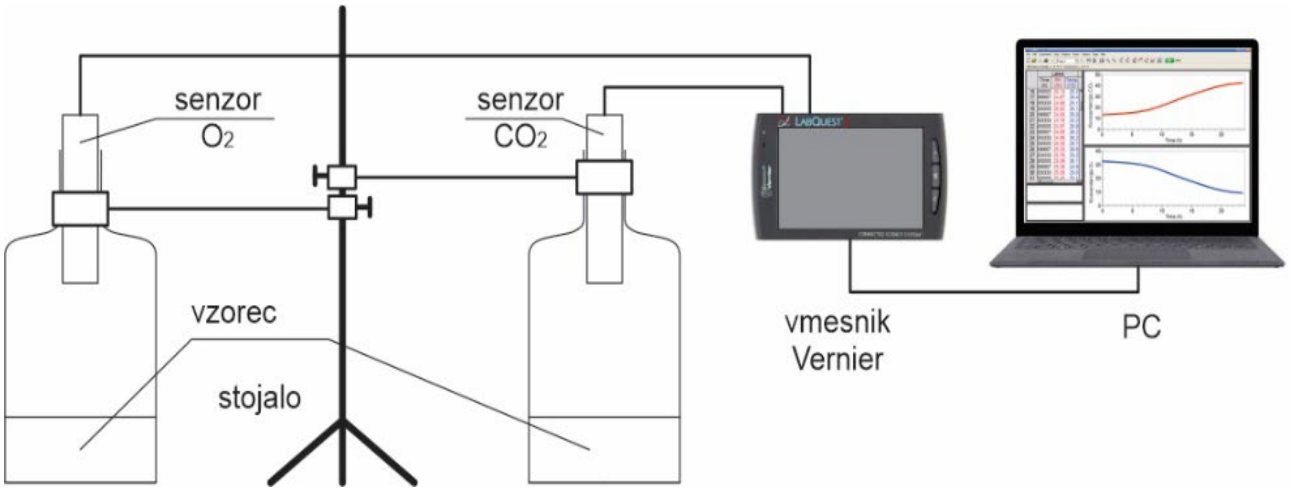

Figure 2: Schematic presentation of Vernier system

Very important: The meter is used to measure gaseous O_2, not O_2 dissolved in a liquid, so do not immerse it in any liquid. When not in use, the meter must be installed vertically. This is important for maintaining the meter!

1. First, take measurements on dry seeds (control 1), then on imbibed seeds treated with 0% (control 2), 1%, 5% and 10% NaCl solutions. Measure the concentrations of gaseous oxygen (O_2) and carbon dioxide (CO_2).
2. Pre-weigh the germinated seeds of each treatment and fill the O_2 tube (chamber) and the CO_2 tube with them (plant material must not be in direct contact with the Vernier gauge).
3. Insert the Vernier meter for O_2 and CO_2 measurement vertically downward into the chamber. Gently secure the chamber to the stand with a clamp.
4. Connect the two meters to the interface, and the interface to the computer (O_2 meter and CO_2 meter). Open the Logger Pro program, set the measurement time, and wait for the program to automatically recognize the two meters and start the measurement. Between each measurement, ventilate the chamber thoroughly for 5–10 minutes).

RESULTS AND OBSERVATIONS

Collect the results of the control and all treatments on one graph, then comment on and explain them.

QUESTIONS

Why are there two controls in this experiment (control 1 and control 2)?

Explain the effect of NaCl on the intensity of respiration.

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6. Exercise:

Photosynthetic Activity

INTRODUCTION

Photosynthetic activity can be determined by measuring O_2 production or CO_2 consumption. CO_2 is transported by diffusion into chloroplasts, where it is incorporated into triose glyceraldehyde and then into glucose through an assimilatory reduction process in the Calvin cycle with the help of the enzyme RUBISCO. Based on differences in photosynthetic carboxylation, the photosynthetic metabolism of C3, C4, and CAM plants can be distinguished. In C4 and CAM metabolism, the first product of CO2 fixation is oxaloacetate, malate, or aspartate, each containing four carbon atoms. These C4 compounds are intermediate CO_2 storage to maintain a high CO_2 partial pressure. By decarboxylation, they are then directed to the Calvin cycle. In all three metabolism pathways, assimilatory reduction of CO_2 occurs via the Calvin cycle (Likar and Vogel-Mikuš, 2011).

OBJECTIVES

- You will study photosynthetic activity by measuring CO_2 consumption under different wavelengths of light (white, red, blue, and green).

TASK

1. Expose common duckweed or lesser duckweed (Lemna minor) to light of different qualities for a set time (20 minutes) and monitor CO_2 consumption under each light condition.

MATERIALS

Common duckweed or lesser duckweed (Lemna minor) plants, gas exchange chamber, carbon dioxide meter, LoggerPro interface, computer with LoggerPro, and light sources (bulbs) of different qualities (white, red, green, and blue).

PROCEDURE

Pour 60 ml of water into the chamber and add 10 g of water lens. Insert a Vernier CO_2 meter, properly connected to a computer. In the program, set the measurement time to 30 minutes and the data acquisition rate to two readings per minute, then start data acquisition.

RESULTS AND OBSERVATIONS

Display the results of CO_2 consumption under different light conditions (white, red, green, and blue) graphically and comment on these.

QUESTIONS

How do you interpret the differences in CO_2 consumption for different spectral properties of light?

NOTES

section 3

ENZYME ACTIVITY

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7. Exercise:

Catalase Activity

INTRODUCTION

Catalase is a widely distributed enzyme found in virtually all aerobic cells, located in peroxisomes and glyoxisomes (in animals, plants, and microorganisms). It belongs to the group of oxidoreductase enzymes. Catalase catalyses the breakdown of hydrogen peroxide (H_2O_2), releasing oxygen (O_2):

$$2\,H_2O_2 \xrightarrow{\text{catalase}} 2\,H_2O + O_2 \qquad (1)$$

It is a relatively stable enzyme that can be easily measured by the amount of oxygen released from the substrate. Catalase consists of four subunits forming a tetramer (1 subunit = 65 kD). Each polypeptide subunit contains a prosthetic group in the active site, which is a hemin (a porphyrin with Fe^{+3}). One iron atom catalyses the reaction of two molecules of hydrogen peroxide. Within a single species, different catalase isozymes can be present. Catalase protects cells from the toxic effects of hydrogen peroxide by breaking it down into molecular oxygen and water.

Hydrogen peroxide is produced as a by-product of metabolism. It is generated in almost every aerobic process in the cell, and particularly in two processes: photorespiration (in peroxisomes) and β-oxidation of fatty acids (in glyoxisomes) during seed germination.

Hydrogen peroxide is toxic to cells because it is a very strong oxidant that would otherwise attack other organic molecules.

QUESTIONS

What are enzymes?

What other groups of enzymes are there?

Which groups of oxidoreductases are known and what do they catalyse?

Which hydrolases are known, how do they work, and what reactions do they catalyse?

You can find out more about enzymes at www.plantphys.net in the "Energy and Enzymes" section.

OBJECTIVES

You will learn how factors such as pH, enzyme concentration, temperature, and substrate concentration (H_2O_2) regulate the enzymatic activity of catalase.

TASK

1. Measure the effect of buffer solutions with pH values of 4, 5, 6, 6.6, 7.2, 7.8, 8.4, 9, 10, and 12 on catalase activity.
2. Plot the reaction rate against the pH of the medium.
3. Measure the effect of catalase enzyme concentration (0.00, 0.25, 0.50, 1.00, 2.00, 3.00, 4.00 ml of enzyme in the sample, always supplemented with buffer at pH = 7.2 up to a total volume of 22 ml) on the rate of the enzymatic reaction.
4. Plot the rate of reaction against enzyme concentration.
5. Measure the effect of the substrate concentration - H_2O_2 (0.0, 0.5, 1.0, 2.0, 4.0, 8.0, 10.0 ml H_2O_2, supplemented, if necessary, with buffer pH = 7,2 to 10 ml) on the catalase activity.
6. Plot the reaction rate versus substrate concentration.
7. Measure the effect of temperature (water bath at 10, 20, 30, 40, 50, 60, 70, 80, 90 ºC) on catalase activity.
8. Plot the reaction rate against the temperature of the water bath.

MATERIAL

Potato tuber or other plant material, 50 ml burette, 60 ml separating funnel, 100 ml reaction bottle with stopper, connecting tubes, mortar and pestle, 0.1 to 10 ml pipettes, hotplate with stirrer.

CHEMICALS

3% H_2O_2, various buffers prepared from 0.1 M citric acid (A), 0.2 M Na_2HPO_4 (B), 0.2 M NaH_2PO_4 (C), 0.2 M KCl (D), 0.2 M H_3BO_3 (E), 0.2 M NaOH (F).

PROCEDURE

Extract the enzyme from 3 g of any plant material (e.g., fresh spinach leaves without stems or finely grated potato tuber). Prepare 50 ml of deionized water. Pour part of the water into a terylene flask, add the finely chopped or grated plant material, and shake well to release the enzyme from the tissue. Add the remainder of the 50 ml deionized water and allow the coarse plant material to settle at the bottom. Decant the extract (supernatant) without sediment into a glass rod and keep it in the bottle for the duration of the experiment.

Assemble the apparatus with the help of the assistant according to the scheme (Figure 3). The water bath can be replaced by a heating plate.

Add the enzyme extract (supernatant) with the appropriate buffer to the reaction vessel and place the H_2O_2-substrate in a separating funnel (see Execution points 1, 2, 3, 4 and 5).

Immerse the reaction vessel in a water bath or place it on a hotplate with a stirrer at 30°C and connect it with a tube to an inverted burette filled with water.

After 5 minutes of thermal equilibration, slowly lower the substrate (H_2O_2) from the separatory funnel into the reaction vessel. Immediately afterwards, close the tap on the separatory funnel to prevent the escape of the oxygen formed. Once the substrate has displaced some of the air in the burette because of physical phenomena, quickly read the water level (mark it), and start measuring the reaction time. Stir the contents of the reaction vessel gently throughout the reaction.

After 10 minutes of reaction, record the volume of oxygen released based on the displaced water (or the time during which 50 ml of oxygen has accumulated if this time is less than 5 minutes).

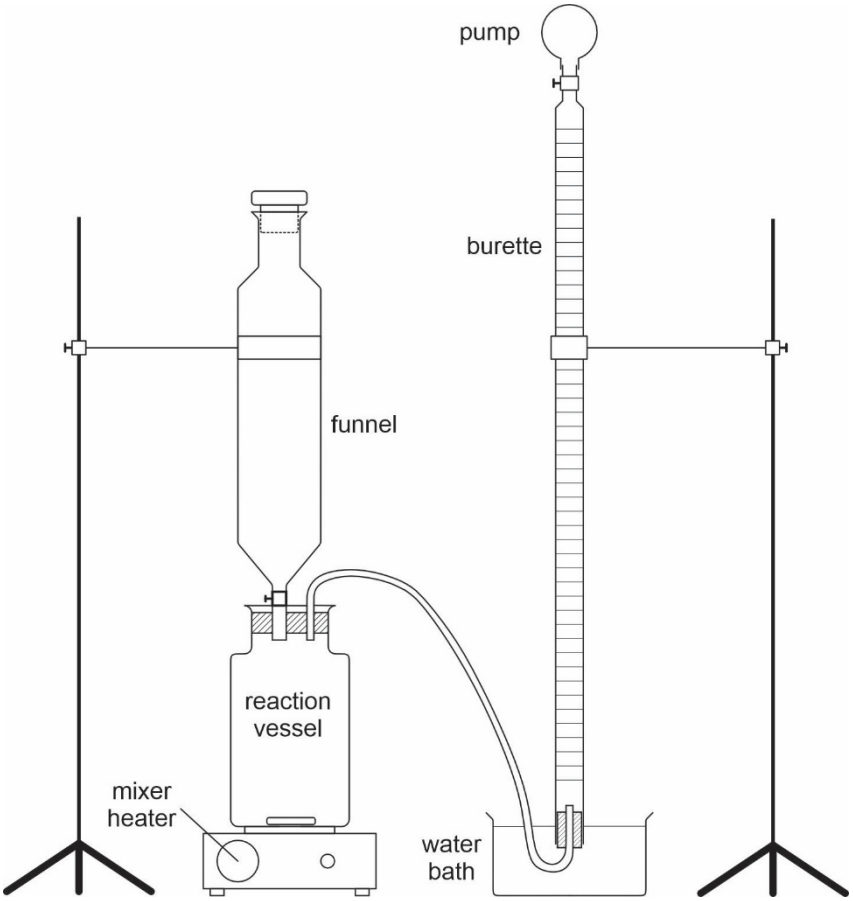

Figure 3: Set-up of the catalase activity meter

Effect of different factors on catalase activity:

1. Effect of pH on the reaction rate: Place 2 ml of enzyme extract (supernatant) and 20 ml of buffer of the appropriate pH (4, 5, 6, 6.6, 7.2, 7.8, 8.4, 9, 10, 12) in a reaction vessel, and 10 ml of H_2O_2 in a separatory funnel. Allow the reaction to proceed for 10 minutes at each pH. After 10 minutes, record the volume of oxygen produced (Table 6).
2. Effect of enzyme concentration on the reaction rate: Place a mixture of enzyme extract and buffer (0.00, 0.25, 0.50, 1.00, 2.00, 3.00, 4.00 ml) of enzyme in the sample, always made up to 22 ml with buffer at pH = 7.2 in a reaction vessel, and 10 ml of H_2O_2 in a separatory funnel. At each indicated enzyme concentration, allow the reaction to proceed for 10 minutes. After 10 minutes, record the volume of oxygen produced (Table 7).
3. Effect of substrate concentration on the rate of reaction: Place 2 ml of enzyme extract (supernatant) and 20 ml of buffer pH = 7.2 in a reaction vessel and appropriately

diluted substrate (0.0, 0.5, 1.0, 2.0, 4.0, 8.0, 10.0 ml of H_2O_2, made up to 10 ml with buffer pH = 7,2 for different dilutions) in a separatory funnel. Allow the reaction to run for 10 minutes at each indicated substrate concentration. After 10 minutes, record the volume of oxygen formed (Table 8).

4. Effect of temperature on the reaction rate: Place 2 ml of enzyme extract (supernatant) and 20 ml of buffer at pH = 7.2 in the reaction vessel, and 10 ml of H_2O_2 in the separatory funnel. Vary the temperature of the water bath or heating plate (T = 10, 20, 30, 40, 50, 60°C). At each temperature, allow the reaction to proceed for 10 minutes. After 10 minutes, record the volume of oxygen produced (Table 9).

Table 5: Preparation of buffers (200 ml)

pH	ml
4,0	61,4 A + 38,6 B
5,0	48,6 A + 51,4 B
6,0	35,8 A + 64,2 B
6,6	62,5 C + 37,5 B
7,2	28,0 C + 72,0 B
7,8	8,5 C + 91,5 B
8,4	50,0 D + 50,0 E + 8,6 F
9,0	50,0 D + 50,0 E + 21,4 F
10,0	50,0 D + 50,0 E + 39,1 F
12,0	50,0 D + 50,0 E + 61,0 F

0,1 M citric acid (A)

0,2 M Na2HPO4 (B)

0,2 M NaH2PO4 (C)

0,2 M KCl (D)

0,2 M H3BO3 (E)

0,2 M NaOH (F)

Each buffer makes up to 200 ml with deionized H_2O.

RESULTS AND OBSERVATIONS

Table 6: Effect of pH on the rate of enzymatic reactions

Buffer pH	Volume O_2 (ml min^{-1})

Table 7: Effect of enzyme concentration on the rate of an enzymatic reaction

Enzyme amount in the sample (ml)	Volume O_2 (ml min^{-1})

Table 8: Effect of sample concentration on the rate of an enzymatic reaction

Volume of H_2O_2 in the sample (ml)	Volume O_2 (ml min^{-1})

Table 9: Effect of temperature on the rate of an enzymatic reaction

Temperature (°C) of buffer solution with the enzyme	Volume O_2 (ml min^{-1})

section 4

WATER UPTAKE, TRANSPORT AND BALANCE IN PLANTS

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8. Exercise:

Specific Conductivity of Wood

INTRODUCTION

Water enters the plant through the root hairs and then moves by radial transport through the root cortex to the endodermis, which acts as a physiological barrier to water in the xylem. It is then transported axially through the vascular tissue of steam to the leaves, where it evaporates from the surface of the mesophyll cells and passes through the leaf stomata and, to a small extent, through the cuticle into the surrounding atmosphere.

The rate of water transport depends not only on transpiration, but also on the structure and function of the conducting elements, namely the xylem. In this exercise, we will examine the conductivity of the secondary xylem (wood) in gymnosperms and angiosperms. The wood of different species varies in both structure and conductivity. Usually, conduction occurs only through the outer growth rings and in some cases (oak, elm, chestnut, etc.) only through the last, youngest growth ring. The ability of wood to conduct water is expressed as specific conductivity.

QUESTIONS

How do coniferous and deciduous woods differ in structure?

What is a ring-porous wood and what is a diffuse.porous wood?

What is microporous wood and what is macroporous wood?

OBJECTIVES

– You will identify differences in the conductivity of wood from different wood types of different species of trees and shrubs.

TASK

1. Measure the volume of coloured liquid that flows through a branch in 30 minutes.
2. Calculate the cross-sectional area of the growth branch.
3. Calculate the specific conductivity (sp) using equation (3):

$$sp = \frac{V \cdot l}{t \cdot S \cdot p} \quad (3)$$

V = volume of fluid flow (m^3)
l = length of the growth ring (m)
t = duration of the experiment (h)
S = branch cross-section (m^2)
p = pressure (Pa)*.

* The water pressure above the pipe is equal to 0.01MPa for each meter of liquid above the branch.

1 at = 760 mm Hg = 1.013 bar = 0.1013 MPa

MATERIALS

Branches from various trees about 1 cm thick, a 1 m long tube, a rubber stopper to fit the glass or metal tube onto the branch, wire, a stand, a vise, a measuring cylinder, a beaker, a funnel, a saw, millimetre paper, a ruler, and scissors.

CHEMICALS

0.05 % methylene blue.

PROCEDURE

Cut branches about 30 cm long and place them in water until the beginning of the experiment to prevent cavitation. Saw a 10 cm long piece from the middle of the branch, ensuring there are no side branches and attach it to the glass tube using a rubber stopper. Clamp the assembly onto the stand so that the branch is in a vertical position (see Figure

4). Fill the tube to a height of 1m with the methylene blue solution and allow the dye to flow through the branch into the beaker. Then measure the volume of liquid that has flowed through using the measuring cylinder. During the experiment, continue adding methylene blue so that the height of the liquid in the tube remains constant. Repeat the measurement twice and record the results (see Table 10). After the measurements, measure the diameter of the branch (wood) and cut it in half lengthwise. Observe the stained and unstained parts and draw conclusions from the stained branches about water released.

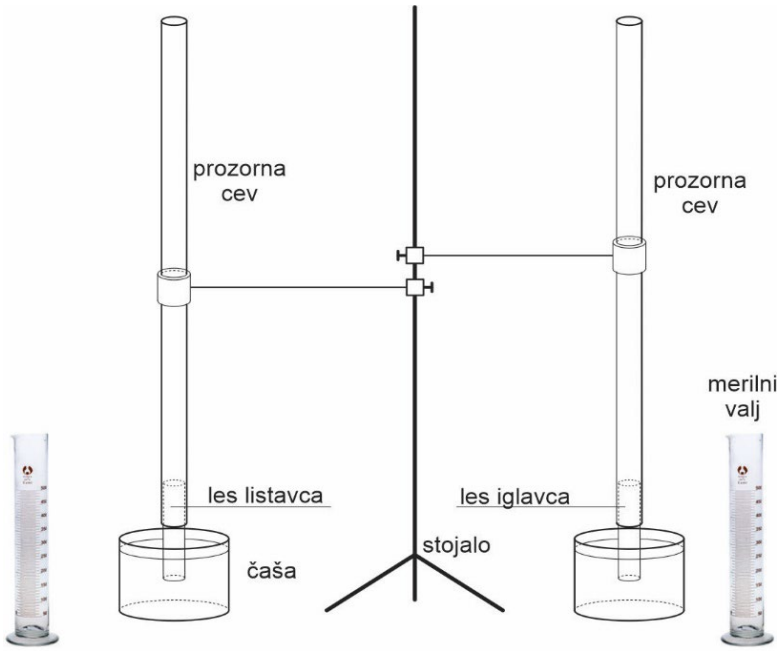

Figure 4: Set of apparatus for measuring the conductivity of wood

RESULTS AND OBSERVATIONS

Table 10: Specific conductivity of different types of wood

Object				
V				
t				
S				
p				
sp				

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9. Exercise:

Succulence

INTRODUCTION

The succulence rate (SS) is a ratio that describes the relationship between the volume and the surface area of the leaf. Many dryland plant species have thick leaves, with a relatively low surface area to volume ratio, a thick cuticle, and very low transpiration. They usually have a poorly developed columnar tissue layer, so most leaves or stems are composed of photosynthetically active spongy cells with large vacuoles and little cytoplasm. CO_2 uptake and photosynthetic metabolism in succulents is of the CAM type (crassulacean acid metabolism). You will learn more about carbon metabolism in plants in the lectures.

QUESTIONS

What are the CO_2 uptake and metabolism processes in C3, C4, and CAM plants?

Which plant groups are characterized by each cycle?

In which habitats are they found?

Through which organs and tissues does transpiration occur?

How do plants regulate transpiration?

How are some plants protected from excessive transpiration?

What are these plants called?

OBJECTIVES

- Learn about the degree of succulence of the leaves of various plant species.

TASK

Calculate the rate of succulence (SS) for each leaf using formula (4).

$$SS = \frac{m}{2 \cdot p} \quad (4)$$

m = mass (content) of water (g) in the leaves
p = leaf area (cm^2).

Determine whether leaf age affects a change in the succulence rate.

MATERIAL

Freshly cut branches with leaves from different plants, Petri dishes, aluminium foil, millimetre paper, scissors, pencil, balance, dryer.

PROCEDURE

Select five different plants and cut branches with at least five leaves (Table 11). Then, from these five plants, choose one and cut the first five leaves from the tip downwards (Table 12). Weigh all the cut leaves.

Measure the surface area of the leaves by drawing them onto millimetre paper. Place the leaves on weighed waxed paper (baking paper) and dry them to a constant weight at 105°C. Weigh the leaves again and determine the water content of all leaves (Table 11, Table 12).

RESULTS AND OBSERVATIONS

Table 11: Succulence rate of different plant species

Leaves of different plant species	Area (cm^2)	Fresh weight (g)	Dry weight (g)	Water content (g)	Succulence rate

Table 12: Effect of leaf maturity on succulence rate

Leaves of different plant species	Area (cm^2)	Fresh weight (g)	Dry weight (g)	Water content (g)	Succulence rate
1st leaf					
2nd leaf					
3rd leaf					
4th leaf					
5th leaf					

NOTES

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10. Exercise:

Water Uptake

INTRODUCTION

The water in a plant is in contact with the water in the soil and in the air. Water in a plant moves from a region of higher to a region of lower water potential (from a less negative to a more negative water potential). Therefore, water transport always occurs from a location of higher water potential in the soil towards a location of lower water potential in the atmosphere, i.e., along the water potential gradient. Since the water potential of relatively dry air is lower than that of the plants by several tens to several thousands of kPa, water in the plant tends to leave the plant.

Water enters the plant through the root hairs and then moves radially along the root cortex, passing through the endodermis, which acts as a physiological barrier/break before reaching the xylem. It then moves into the stem and leaves, where it evaporates from the surface of the mesophyll cells and then diffuses out of the plant through the leaf stomata and, to a lesser extent, through the cuticle. The movement of water through the plant is called transpiration, and the loss of water from the plant surface is also called transpiration. Transpiration occurs mainly through the leaves, specifically through the stomata, and to a lesser extent through the cuticle (cuticular transpiration) and the lenticels of the bark (peridermal transpiration).

You will learn more about water potential, its components, and transpiration in the lectures.

QUESTIONS

What external (environmental) and internal (structural, metabolic) factors affect transpiration?

The device used to measure water uptake in a plant shoot or young plant is a **potometer** (Figure 5). Transpiration is not measured directly but indirectly, since the plant loses most of the water taken up through transpiration. Transpiration and water uptake are therefore closely related.

Transpiration is measured in the following two ways:

Directly - by measuring the volume of water collected in a measuring tube per unit of time. It is assumed that the plant takes up water through the cut part or through the roots, which replace the water lost through transpiration.

Indirectly - by measuring the decrease in the mass of the potometer per unit of time.

Although numerous metabolic processes in the plant (respiration, photosynthesis) also influence the water content and affect the weight of the plant, their effects are negligible compared to transpiration and are not taken into account.

There are two things to consider when setting up a potometer:

- the stem of the plant must be cut under water to prevent air from entering the veins.
- all components must be tight so that the water escapes only by transpiration through the plant.

OBJECTIVES

- You will investigate the influence of wind, humidity and leaf area on water uptake and transpiration.

TASK

1. Construct a sweat meter with the help of an assistant (Fig. 5).
2. Measure the water uptake ($cm^3\ h^{-1}$) of the plant by reading the water consumption in a measuring tube (1-ml pipette).
3. Calculate the water loss per leaf area ($cm^3\ h^{-1}\ m^{-2}$).

MATERIAL

Young leaf shoots of various plants, homemade potentiometer, large transparent plastic bag, electric hair dryer; stopwatch, thermometer, syringe, millimetre paper.

CHEMICALS

Wax or petroleum jelly.

PROCEDURE

1. Water intake

Prepare everything for the assembly of the potentiometer (Figure 5).

Select a plant, cut it and immediately immerse the cut end in water. This will prevent air bubbles from entering the xylem. Under water, a few cm above the first cut, immediately cut the shoot again smoothly and at an angle. While it is still in the water, insert it into the pierced plug of the potometer. Fill the potometer with water and place it in the water basin. Place the 1 ml pipette with the dipstick and syringe in the water. Take the assembled potentiometer out of the water, clamp it in the stand and check the water column in the capillary (pipette). The potentiometer is ready for measurement when the water column in the capillary starts to move in the direction of the water column. Before each measurement, set the capillary pressure with the syringe, and then read the water consumption every 10 minutes over a period of 30 minutes (or adjust the time to what is happening). Results should be calculated as volume per unit of time ($cm^3\ h^{-1}$) (Table 14).

After each change in the experimental conditions, you must adjust (change over) the potentiometer.

Perform a series of four measurements and record the results (Table 13):

a) Control measurement.
b) Wind: simulate this by blowing the plant very gently with a hairdryer (in strong winds, the slits close).
c) Increase humidity: increase the humidity by covering the shoot with a plastic bag.
d) Reduce the surface area by half: reduce the leaf area by half by removing half of the leaf surface.

2. Transpiration

Measure leaf area by removing all leaves from the shoot after completing measurements and calculating their leaf area. Do this by drawing the leaves on millimetre paper and counting the squares on the paper. Report the results in terms of water consumption per unit of time and per leaf area ($cm^3h^{-1}m^{-2}$) (Table 15).

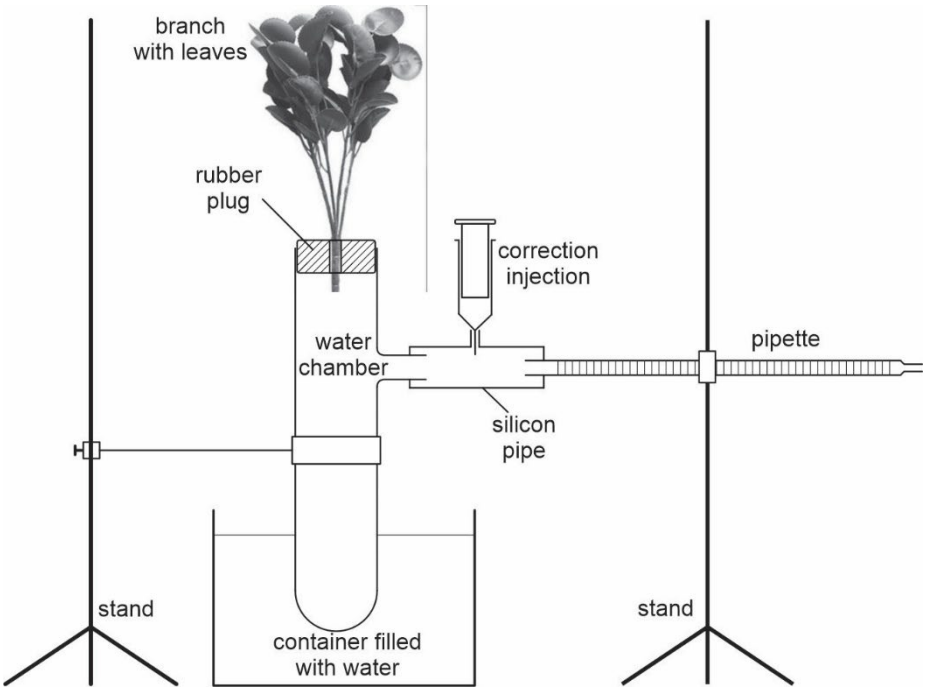

Figure 5: Potometer

RESULTS AND OBSERVATIONS

Table 13: Impact of environmental factors on water consumption

Water consumption (ml)				
Time (min)	Control	Wind (fen)	Humidity (bag)	1/2 leaf surface

Table 14: Water uptake under different environmental factors

Average water consumption ($cm^3\ h^{-1}$)			
Control	Wind (hairdryer)	Humidity (bag)	1/2 leaf surface

Table 15: Water transpiration under different environmental factors

Average water loss per leaf area ($cm^3\ h^{-1}\ m^{-2}$)			
Control	Wind (hairdryer)	Humidity (bag)	1/2 leaf surface

Draw the water consumption and transpiration time trajectories.

section 5

DISPERSION SYSTEMS, COLLOIDS, AND PLASMOLYSIS

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J. Ambrožič-Dolinšek, T. Ciringer

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11. Exercise:

Colloidal Systems

INTRODUCTION

Many compounds in living organisms exist in a colloidal state. Their two most important properties are that they carry an electric charge and they swell, binding ions or molecules with a different electric charge to their surface (lyophilic colloids).

The colloidal solution inside the cell is the cytoplasm. The cytoplasm is a colloidal solution with a size of 1–1000 nm, that fills the interior of cells and is divided into two components: the liquid fraction, called the cytosol or cytoplasmic matrix, and the organelles it contains in eukaryotic cells.

The cytosol is the cell's gelatinous colloidal solution (sometimes colourless, sometimes a greyish substance), and consists of a large amount of solutes such as ions, intermediate metabolites, carbohydrates, lipids, proteins, and ribonucleic acids (RNA). It can exist in two interchangeable phases: as a gel or as a sol.

The main elements are carbon, hydrogen, nitrogen, oxygen, phosphorus, and sulphur.

The cytosol is also rich in ions, which increases the osmotic pressure in the cell and help maintain an optimal acid-base balance in the cellular environment.

The variety of ions contained in the cytosol depends on the cell type. For example, muscle and nerve cells have high concentrations of potassium and magnesium, while calcium ions are especially abundant in blood cells.

In plant cells, colloids in which the dispersant is water (hydrophilic colloids) predominate. When they swell, water dipoles bind to macromolecules, which are usually proteins but can also be polysaccharides.

The operation of such colloidal systems is illustrated by an exercise in which a dispersion system is prepared from agar (a polysaccharide with a negative charge on the macromolecules) and water or various ionic solutions. Negatively charged agar swells in water because water molecules are bound to its surface.

QUESTIONS

What are dispersive systems?

Which of these are colloidal systems?

What is a dispersed phase and a dispersant?

How are the concentrations of the solutions below prepared?

How are they calculated?

What data do we need?

What are molarity and molar concentration?

OBJECTIVES

- You will use a model to simulate the processes in a plant cell resulting from the properties of hydrophilic colloids.

TASK

1. Determine the effect of added ions on the swelling of the agar as a function of the combination of added ions, their electric charge, ion size, and hydration shell.

MATERIALS

Six test tubes of equal diameter, 25 ml beaker, larger beaker, spoon, balance, test tube stand.

CHEMICALS

Agar, 1 M solutions of KI, KBr, KCl, NaCl, LiCl, deionized water.

PROCEDURE

1. Carefully shake 0.5 g of agar into each of the six identical tubes (Figure 6).
2. Fill five tubes with 10 ml each of the solutions (KI, KBr, KCl, NaCl, LiCl) and the sixth tube with the same volume of distilled water. Immediately label the tubes with the type of solution.
3. Place the tubes in a hot water bath. After one hour, check the degree of agar swelling and record your observation.

RESULTS AND OBSERVATIONS

Record (photograph) the level of swollen agar in each tube and explain the results (Figure 6).

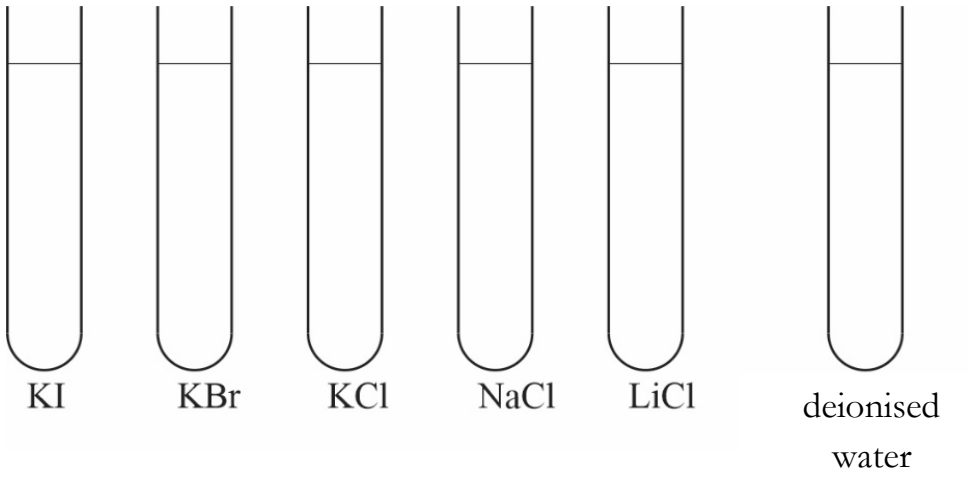

Figure 6: Effect of ions (KI, KBr, KCl, NaCl, LiCl) and water in a colloidal system

NOTES

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12. Exercise:

Plasmolysis

INTRODUCTION

Plasmolysis is the detachment of the cell membrane from the cell wall, owing to shrinkage of the cytoplasm and the vacuole following the loss of water in a hypertonic environment (an environment with a higher concentration of solutes).

Plant cells are normally in a turgid state. Turgor enables nutrient solutions to move between cells, maintains plant rigidity, and keeps the plants upright.

In a cell with an adequate amount of water, the cell membrane presses against the cell wall and is in perfect contact with it. When this cell is placed in a hypertonic solution, water begins to flow out of the cell, initially without affecting the cell wall. However, as water continues to be lost from the cell because of the hypertonic environment, the first signs of shrinkage in the cell contents appear - the initial phase of plasmolysis, where the cell membrane is detached from the cell wall at the ends but still maintains contact in other areas.

In the second phase, as the cell continues to lose water under hypertonic conditions, the cell membrane completely detaches from the cell wall, shrinks to a spherical shape and remains in the centre of the cell.

As plasmolysis progresses, the contraction of the cell and cytoplasm reaches a minimum, and further volume reduction is no longer possible. Depending on the final shape of the cytoplasm, final plasmolysis is divided into two types: concave plasmolysis and convex plasmolysis.

In concave plasmolysis, the protoplasm and cell membrane shrink and separate from the cell wall because of water loss. As the protoplasm begins to detach from the cell wall, it transforms into a protoplast. This process can be reversed if the cell is placed in a hypotonic solution, causing water to flow back into the cell.

Convex plasmolysis occurs when the cell membrane and protoplast lose so much water that they completely detach from the cell wall. Convex plasmolysis cannot be reversed and results in the destruction of the cell. The plant withers and dies for lack of water.

In the laboratory, plasmolysis can be demonstrated by placing a living cell in a sodium chloride solution or another salt solution in which the water concentration inside the cell will be higher than outside the cell. Therefore, water migrates out of the cell through the cell membrane into the surrounding medium. Finally, the protoplasm detaches from the cell wall and assumes a spherical shape, resulting in plasmolysis.

When a plasmolyzed cell is placed in a hypotonic solution (a solution in which the solute concentration is lower than that of cell sap), water enters the cell because of the higher water concentration outside the cell. The cell then swells and regains its turgor. This process of restoring the normal turgor of a plasmolyzed cell is called **deplasmolysis**.

QUESTIONS

What is meant by diffusion, osmosis, osmolarity, tonicity, plasmolysis, and water potential of a cell?

Repeat: cell wall, plasmalemma, cytoplasm, tonoplast, vacuole, protoplasm.

OBJECTIVES

- You will learn about the behaviour of cells in solutions with different water potentials.
- You will learn about plasmolysis: types of plasmolysis, marginal plasmolysis and deplasmolysis.

TASK

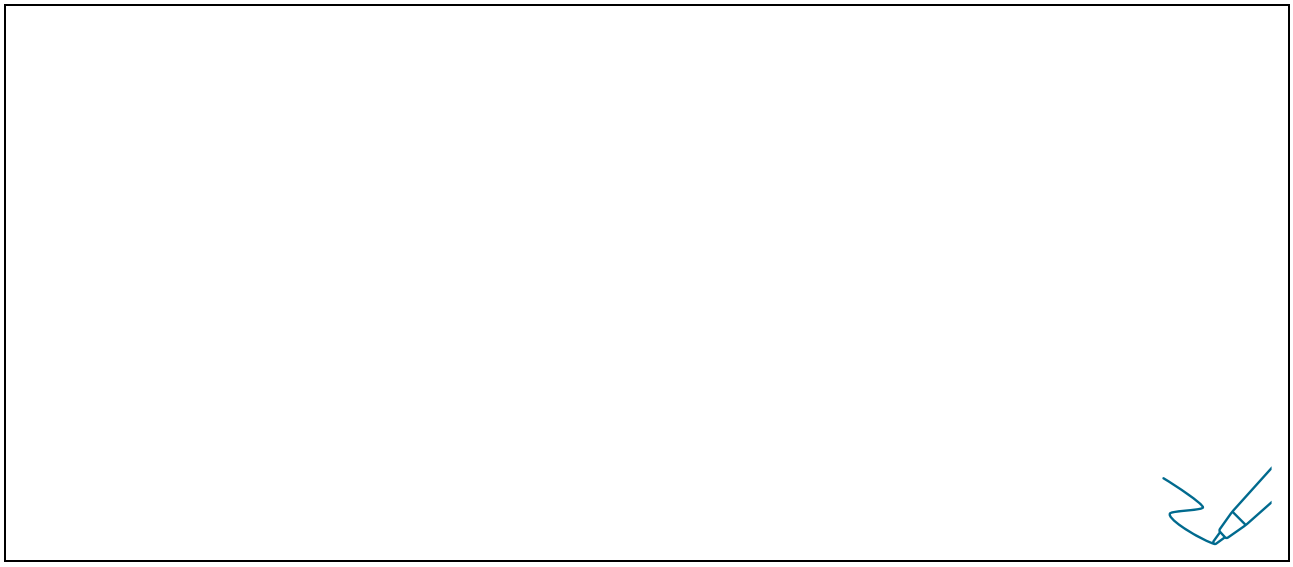
Observe and draw the stages of plasmolysis:

1. Incipient plasmolysis,

2. Concave plasmolysis (low-viscosity protoplasm),

3. Convex plasmolysis (high-viscosity protoplasm),

4. Cap plasmolysis (tonoplast and plasmalemma are not equally permeable to osmosis).



MATERIAL

Red onion (*Allium cepa*), microscope and accessories, razor blade or scalpel, filter paper.

CHEMICALS

1 M KNO_3 solution, 0.7 M $Ca(NO_3)_2$ solution, 1 M sucrose solution, 1 M KSCN solution.

PROCEDURE

1. Using a razor blade, cut a square approximately 5 x 5 mm from the top of the fleshed onion base. Carefully peel off the epidermis with forceps, place it on a glass slide in a drop of the chosen hypertonic solution, cover with a coverslip and immediately observe the changes in the protoplast of the cell under a microscope. Observe until plasmolysis has stopped and is no longer progressing. Record the stages of plasmolysis. Work stepwise: prepare one slide at a time, observe it, draw it, and then prepare the next slide. You will prepare four slides:

    a) Place the tissue in a drop of hypertonic KNO_3 solution, immediately observe the changes in the protoplast of the cell and draw each stage of plasmolysis.
    b) Place the tissue in a drop of hypertonic $Ca(NO_3)_2$ solution, immediately observe the changes in the protoplast of the cell, and draw the individual stages of plasmolysis.
    c) Place the tissue in a drop of hypertonic sucrose solution, immediately observe the changes in the protoplast of the cell and record the individual stages of plasmolysis.
    d) Place the tissue in a drop of hypertonic KSCN solution, immediately observe the changes in the protoplast of the cell and draw each stage of plasmolysis.

2. Add distilled water to the coverslip under which the tissue lies in one of the above solutions and draw it to the other side using filter paper. Observe the changes in the protoplast of the cell and explain and define what is happening.

RESULTS AND OBSERVATIONS

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13. Exercise:

Measuring Water Potential by Incipient Plasmolysis

INTRODUCTION

You have learned most of the theory of plasmolysis and water potential in the lectures. Review and explain the theoretical principles by answering the questions below.

QUESTIONS

What is the water potential of a cell?

What is osmoticity?

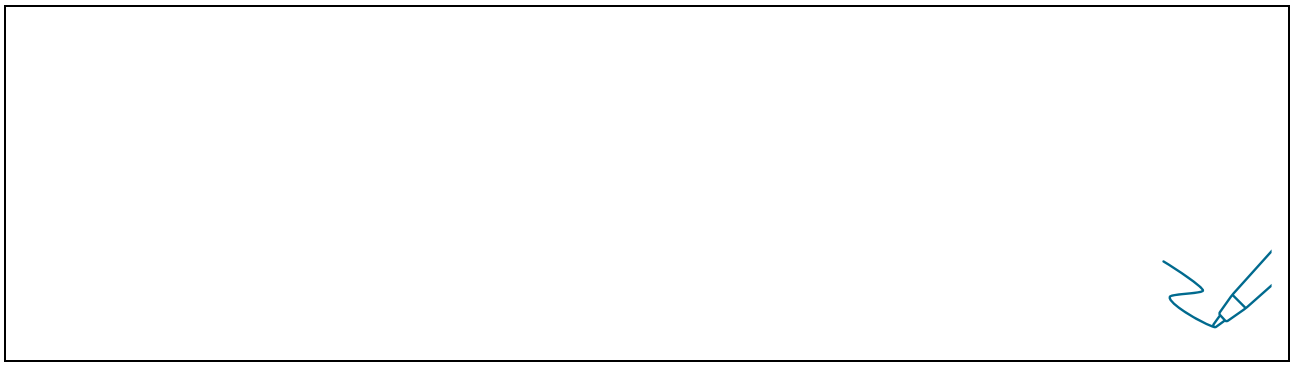
What is tonicity?

When is a cell (or tissue) iso-, hypo-, or hypertonic compared to the solution in which it is immersed?

When does incipient plasmolysis begin in a cell, and what is the water potential of the cell and the water potential of the surrounding solution at that time?

How are different plasmolytic solutions prepared?

What data do we need and how would we calculate this?



What does molarity or molar concentration mean?

There are several methods for determining the water potential of cells or tissues. One of the most common methods is by **incipient plasmolysis**. In this case, the water potential of the cell (ψ_c) is equal to the water potential of the solution (ψ_r) in which the cell is located. At this point, ψ cells = ψ solutions. The solution in which incipient plasmolysis has started has almost the same (slightly lower) water potential as the solution inside the cell.

OBJECTIVES

- You will measure the water potential of a cell by determining the point of incipient plasmolysis.

TASK

1. Place onion leaf tissue or cells in a series of plasmolytic solutions of increasing concentration and determine in which solution incipient plasmolysis occurs. Incipient plasmolysis occurs between the solution in which the cell's protoplast begins to detach from the cell wall and the solution of lower concentration.
2. Calculate the water potential of the solution using the threshold plasmolysis data.

MATERIALS

Red onion (*Allium cepa*), 12 slides and coverslips, razor blade or scalpel, tweezers, coverslips, microscope.

CHEMICALS

KNO_3 (or sucrose) plasmolytic solutions: 0.00 M, 0.05 M, 0.10 M, 0.15 M, 0.20 M, 0.25 M, 0.30 M, 0.35 M, 0.40 M, 0.45 M, 0.50 M, 0.60 M.

PROCEDURE

1. Place two drops of each solution from the series at different concentrations on each slide.
2. Place a piece of cuticle from an onion skin on each slide and cover it with a coverslip.
3. Using a microscope, determine at which concentration the incipient plasmolysis has occurred. If the plasmolytic agent is KNO_3, plasmolysis is observed immediately, but if the plasmolytic agent is sucrose, plasmolysis is observed after about half an hour. (Why?) The solution in the cell is isotonic and lies between the solution where incipient plasmolysis occurs and the solution with the next lower concentration. Calculate the water potential of the solution using equation (5):

$$\psi_r = - c \cdot i \cdot R \cdot T \qquad (5)$$

ψr = water potential of the solution (1 Pa = N m^{-2}, N = Newton);
c = molar concentration of the solution (mol l^{-1});
i = isosmotic constant; for sucrose i = 1; for KNO_3 i = 1.69;
R = universal gas constant (8.3 J mol^{-1} K^{-1}; J = N m);
T = temperature in kelvin (T = 0 °C = 273 °K)
1 bar = 0.987 atm = 105 Pa = 100 kPa

1000 kPa = 1 MPa.

RESULTS AND OBSERVATIONS

The water potential (ψ) consists of the solution potential (ψ_r) and the pressure potential (ψ_p). Why did you not take the pressure potential into account in your measurements?

section 6

MINERAL NUTRITION OF PLANTS

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J. Ambrožič-Dolinšek, T. Ciringer

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14. Exercise:

Symptoms of Physiological Disorders Following Deficiency in Certain Nutrients

INTRODUCTION

Autotrophic nutrition involves not only the synthesis of carbohydrates from CO2 and H+, with water as a donor, but also the synthesis of other organic substances that require mineral elements such as N, S, P, K, and Ca. Some of these elements are required by plants in large quantities (>0.1%) and are called macronutrients (N, O, H, C, P, S, K, Ca, Mg), while others are required in very small quantities (<0.1%) and are called micronutrients (Fe, B, Mn, Zn, Cu, Mo, Co).

Deficiency in a particular element manifests in symptoms such as stunting, yellowing (chlorosis – collapse of chlorophyll), browning (necrosis – collapse of mesophyll), or other signs. Symptoms of deficiency in elements that are well transported through the plant are evident throughout the plant (N, P) or in older parts, such as older leaves (K, Mg). Symptoms of elements that are poorly translocated appear mainly in young plant parts, such as shoots and young leaves (S, Ca, Fe, B, Mn).

You will learn more about mineral nutrition in the lectures.

OBJECTIVES

– Using the technique of hydroponics (growing plants in nutrient solutions with known amounts of mineral elements), you will study the growth and development of plants in complete nutrient solutions and in nutrient solutions from which one or more elements have been removed and observe the symptoms of specific nutrient deficiencies.

TASK

1. Prepare individual nutrient water solutions. Measure the pH of the nutrient solutions.
2. Prepare your plant material. Measure the length of the whole plant, the shoot, and the main root, and record the number of leaves.
3. Place the plant in the prepared nutrient solution.
4. Replace evaporated water or add the appropriate nutrient solution every week for the duration of the experiment (two months).
5. Every two weeks, observe and describe deficiency symptoms, measure the length of the whole plant, the shoot and the main root, and record the number of leaves.
6. At the end of the experiment, measure the pH of the nutrient solution.

MATERIAL

Young tomato plants, one-liter jars wrapped in aluminium foil, labels, pencil or alcohol marker, pH indicator, lids with two small openings and one large opening.

CHEMICALS

1 M $Ca(NO_3)_2$, 1 M KNO_3, 1 M $MgSO_4$, 1 M KH_2PO_4, Na-FeEDTA[*1],
1 M $NaNO_3$, 1 M $MgCl_2$, 1 M Na_2SO_4, 1 M $_{NaH2PO4}$, 1 M $CaCl_2$ 1 M KCl, micronutrients solution*[2].

Preparation of the solutions:

1. [*1] Prepare Na-FeEDTA: dissolve 5.57 g $FeSO_4 \cdot 7H_2O$ in 200 ml of deionized water. Then dissolve 7.45 g Na_2EDTA in 200 ml of deionized water. Warm both solutions, mix them while warming, then cool and dilute with water to a final volume of 1000 ml.

2. [*2] Preparation of microelements: dissolve 2.86 g H_3BO_4, 1.81 g $MnCl_2 \cdot 4H_2O$, 0.11 g ZnCl2, 0.05 g $CuCl_2 \cdot 2H_2O$ and 0.025 g $Na_2MoO_4 \cdot 2H_2O$, then make up to 1000 ml.

PROCEDURE

1. Fill each glass jar to half the intended volume with deionized water, then pipette each ionic solution as specified in Table 16 and make up to the calibration mark with deionized water. Measure the pH of each solution.
2. Wrap each glass jar in aluminium foil and label it with the missing element, the control complete solution (kit), or deionized water.
3. Rinse the soil from the roots of the selected plants under running water and measure all required parameters (Table 17).
4. Insert each sample into the largest opening of the lid so that it is stabilized with cotton wool at the point of contact with the lid. The cotton wool should remain dry throughout the experiment.
5. Close the jar with the stopper and place it in the experimental area in the classroom. Maintain the level of the nutrient solution by refilling as needed. Take measurements and observations every two weeks and record all data (Table 17). Take care not to damage the plants during the process.

Table 16: Composition of nutrient solutions

	ml									
Nutrient solution (1M)	**Complete**	**- Ca**	**- S**	**- Mg**	**- K**	**- N**	**- P**	**- Fe**	**- Microelements**	**Deionized. H_2O**
$Ca(NO_3)_2$	10	-	10	10	10	-	10	10	10	-
KNO_3	10	10	10	10	-	-	10	10	10	-
$MgSO_4$,	4	4	-	-	4	4	4	4	4	-
KH_2PO_4	2	2	2	2	-	2	-	2	2	-
NaFeEDTA	2	2	2	2	2	2	2	-	2	-
Microelements	2	2	2	2	2	2	2	2	-	-
$NaNO_3$	-	20	-	-	10	-	-	-	-	-
$MgCl_2$	-	-	4	-	-	-	-	-	-	-
Na_2SO_4	-	-	-	4	-	-	-	-	-	-
NaH_2PO_4	-	-	-	-	2	-	-	-	-	-
$CaCl_2$	-	-	-	-	-	10	-	-	-	-
KCl	-	-	-	-	-	10	2	-	-	-

RESULTS AND OBSERVATIONS

Table 17: Nutrition solution_____ and deficiency symptoms

Date	pH of the nutrition solution*	The length of shoots (cm)	The length of roots (cm)	Deficiency symptoms

* Measure at the beginning and at the end of the experiment.

QUESTIONS

What is the significance of pH in a nutrient solution? Does the pH of nutrient solutions change during the experiment? Why?

Why do deficiency symptoms of some elements appear first in older leaves, while for other elements they appear first in younger leaves?

Why do some symptoms appear earlier than others?

Using the literature, complete Table 18.

Table 18: Symptoms of deficiency

MACROELEMENTS			
Elem.	Form of acceptance	Where can we find it?	Symptoms of deficiency
N	NO^{3-} NH_4^+	proteins, nucleic acids, enzymes, chlorophyll, nucleotides,	
P	PO_4^{3+} H_2PO^{4-}	proteins, DNA, GTP, ATP, FAD, NADP, lipids	
K	K^+	associated with membrane functions, cofactor in photosynthesis and respiration, in the cytoplasm in ionic form, also bound to proteins	
S	SO_4^{2-}	protein synthesis (cysteine, cystine, methionine), Coenzyme A, –SH group in enzymes	
Ca	Ca^{2+}	in the central lamella of the cell wall (protopectin network), bound in organelles, scarce in cytoplasm	
Mg	Mg^{2+}	electron acceptor in chlorophyll – photosynthesis, structural building block of the central lamella, ionic bond between pectin molecules, linking of ribosomal subunits	
Fe	Fe^{3+}	involved in chlorophyll biosynthesis (eventually replaced by Mg), photosynthesis (ferredoxin), respiratory chain (cytochrome oxidase),	

MICROELEMENTS			
Elem.	Form of acceptance	Where can we find it?	Symptoms of deficiency
Mn		cofactor in enzymes (phosphatases, auxin oxidase)	
Cu		cofactor in enzymes (cytochrome oxidase), respiratory chain (PQ)	
Zn		anaerobic respiration (alcoholic fermentation), tryptophan biosynthesis	
Mo		cofactor in enzymes (nitrate reductase)	
B		normal cell division of meristems, NK biosynthesis	
Co		cofactor in vitamin B12	

section 7

REDUCING SUGARS

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15. Exercise:

Qualitative Assessment of Reducing Sugars

INTRODUCTION

Reducing sugars are aldoses and ketoses with a free reactive -OH group that is not blocked by a glycosidic bond. Reducing sugars include all monosaccharides such as glucose and fructose, and some disaccharides such as maltose, lactose, and cellobiose. Sucrose is not a reducing sugar.

QUESTIONS

Where in the metabolism of plant cells are reducing sugars produced?

Where and in what form are sugars transported through the plant from the point of production to the point of consumption, and how is this transport accomplished?

In what form are they stored by the plant?

In which cell organelles and plant organs do plants store reserve sugars?

At what time of year are most of the sugars in storage form?

How do the sugars pass through the cellulose cell wall and the plasmalemma?

OBJECTIVES

- To identify the presence of reducing sugars in leaves.
- To become familiar with the Fehling test for reducing sugars.

TASK

1. Determine the presence of reducing sugars in grass leaf extract and onion peel extract and compare them (take a photo).

MATERIAL

Plant material (green grass, onions, etc.), water, mortar and pestle, filter paper, funnel, small beaker, large test tube, burner, tongs.

CHEMICALS

Fehling reagent I. (3,5 g $CuSO_4 \times 5H_2O$ + 100 ml deionized H_2O) and Fehling reagent II. (18 g $C_4H_4O_6NaK$ + 6 g NaOH + 100 ml deionized H_2O).

PROCEDURE

The grass and the scale leaves of the onion are each steamed separately in a heat chamber. Add a few millilitres of water and filter the extract.

Fehling test: Place about 2 mL of the extract (2 fingers) in a test tube and add first 1 mL of Fehling reagent I (1/2 finger), then the same volume of Fehling's reagent II and heat over a flame until a precipitate forms.

The colour of the final precipitate varies from green, yellow, or orange to red-brown, depending on the amount of reducing sugars. (The initial yellow colour of the precipitate, together with the blue colour of the copper sulphate, gives a green colour.)

Fehling reagent contains copper sulphate ($CuSO_4$). Reducing sugars reduce soluble and blue-coloured copper sulphate (modra gallica, Slo.) containing copper (II) ions (Cu^{2+}) to insoluble reddish-brown copper oxide (CuO_2) containing copper (I) in a different ionic form (Cu^{+}). The solution can react violently when heated, so use caution: turn the tube away from yourself and others. The test is semi-quantitative, meaning that a rough estimate of the amount of reducing sugars is possible.

RESULTS AND OBSERVATIONS

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16. Exercise:

Quantitative Assessment of Reducing Sugars

INTRODUCTION

An alternative to the Nelson-Somogyi method for the quantitative determination of reducing sugars is the DNS (dinitro salicylic acid) reagent method. It is simple, sensitive, acceptable and suitable for processing a large number of samples in a given time (Sadasivam & Manickam, 2007, p. 6).

OBJECTIVES

- To determine the amount of reducing sugars present in your chosen sample using a calibration curve and a standard graph.

MATERIALS

Spectrophotometer (Spectro Vis Plus), LoggerPro3 software, computer, cuvettes, cotton wool, distilled water blower, 1000 ml beaker for waste liquid, test tubes, test tube rack, pipettes, tips, flasks with caps, beakers, funnel, filter paper, mortar and pestle, knife, cutting mat, water bath (100 ml beaker filled with water to the 500 ml mark), cooker, analytical balance, glass rods, dosing spoons, paper towels, alcohol markers; plant material (e.g., seed germs, tree fruit, tubers, etc.).

CHEMICALS

Distilled water (H_2O), glucose powder ($C_6H_{12}O_6$), fructose powder ($C_6H_{12}O_6$), 3,5-dinitrosalicylic acid ($C_7H_4N_2O_7$), sodium hydroxide (NaOH crystalline phenol (C_6H_5OH), sodium sulphite (Na_2O_3S), potassium-sodium tartrate ($C_4H_4KNaO_6 \cdot 4H_2O$).

Preparation of 3.5-dinitro salicylic acid reagent (DNS) reagent

Weigh 1 g of 3.5-dinitrosalicylic acid into a 50 ml beaker, 200 mg of crystalline phenol into a second baker and 50 mg of sodium sulphite into a third. Label the beakers with an alcohol marker. Weigh 1 g of sodium hydroxide into a 100 ml beaker and make up to 100 ml with distilled water. Mix well to obtain a 1% NaOH solution. Pour some of this solution into each beaker containing the chemicals and mix thoroughly. Combine the contents of all three beakers in a 100 ml flask. Rinse each beaker with 1% NaOH solution and add the rinsings to the flask. Add the remaining 1 % NaOH to the 100 ml flask. Label the flask with "DNS" and the date. If the solution is stored for an extended period, sodium sulphite may be added during use, as sodium sulphite deteriorates the reagent during prolonged storage (Table 18).

Preparation of a 40% Rochelle salt solution (potassium sodium tartrate): weigh 20 g of potassium sodium tartrate into a 50 ml beaker, make up to 50 ml with distilled water, and mix well. Store the solution in a 5 ml flask labelled "40% Rochelle salt" and the date (Table 19).

Table 19: Preparation of 3.5-dinitro salicylic acid reagent (DNS)

Chemicals		Amount
1-% NaOH solution	sodium hydroxide	1 g
	deionized water	up to 100 ml
Dinitro salicylic acid		1 g
crystalline phenol		200 mg
sodium sulphite		50 mg
40-% m Rochelle salt	potassium-sodium tartrate	20 g
	deionized water	up to 50 ml

TASK

1. Prepare standard solutions of glucose, followed by a range of glucose concentrations.
2. Measure the absorbance of each glucose concentration and plot a calibration curve for glucose.

3. Using the measured absorbance from the extracts of the selected samples, determine the glucose content from the calibration curve.
4. Prepare standard solutions of fructose, followed by a range of fructose concentrations.
5. Draw a calibration curve for each fructose based on the measured absorbance of each fructose concentration.
6. Determine the fructose content from the calibration curve using measured absorbance of the extracts from the selected samples.

PROCEDURE

Preparation of the calibration curve

Glucose stock standard solution (1 mg ml^{-1})

Weigh 100 mg of glucose into a 100 ml volumetric flask, make up to the 100 ml mark with distilled water and mix well.

Fructose stock standard solution (1 mg ml^{-1})

Weigh 100 mg of fructose into a 100 ml volumetric flask, make up to the 100 ml mark with distilled water and mix well.

Preparation of glucose standard solutions

Prepare eight 50 ml flasks and label them Ag to Hg. Pipette the following volumes of glucose standard solution into each flask: 2.0, 4.0, 6.0, 8.0, 10.0, 12.0 14.0 and 16.0 ml. Make up each flask to the 50 ml mark with distilled water and shake well (see Table 20).

Glucose standard solutions with mass concentrations of 0.04 mg ml-1, 0.08 mg ml-1, 0.12 mg ml-1, 0.16 mg ml-1, 0.20 mg ml-1, 0.24 mg ml-1, 0.28 mg ml-1 and 0.32 mg ml-1 are prepared in this way (Table 20).

Table 20: Preparation of standard glucose solutions

Erlenmeyer flask	Ag	Bg	Cg	Dg	Eg	Fg	Gg	Hg
Volume of standard glucose stock solution (ml)	2,0	4,0	6,0	8,0	10,0	12,0	14,0	16,0
Volume of distilled water (ml)	48,0	46,0	44,0	42,0	40,0	38,0	36,0	34,0
Glucose concentration by mass γg (mg ml^{-1})	0,04	0,08	0,12	0,16	0,20	0,24	0,28	0,32

Preparation of fructose standard solutions

Prepare eight 50 ml flasks and label them Af to Hf. Pipette the appropriate volume of fructose solution into each flask, namely 2.0, 4.0, 6.0, 8.0, 10.0, 12.0 14.0 and 16.0 ml (Table 21). Make up flasks to the 50 ml mark with distilled water and shake well. Thus, fructose standard solutions with mass concentrations of 0.04 mg ml^{-1}, 0.08 mg ml^{-1}, 0.12 mg ml^{-1}, 016 mg ml^{-1}, 0.20 mg ml^{-1}, 0.24 mg ml^{-1}, 0.28 mg ml^{-1} and 0.32 mg ml^{-1} are obtained (Table 21).

Table 21: Preparation of standard fructose solutions

Erlenmeyer flask	Ag	Bg	Cg	Dg	Eg	Fg	Gg	Hg
Volume of standard fructose stock solution (ml)	2,0	4,0	6,0	8,0	10,0	12,0	14,0	16,0
Volume of distilled water (ml)	48,0	46,0	44,0	42,0	40,0	38,0	36,0	34,0
Fructose concentration by mass γg (mg ml^{-1})	0,04	0,08	0,12	0,16	0,20	0,24	0,28	0,32

Preparation of glucose solutions for spectrophotometer measurement

Pour 3 ml of distilled water and 3 ml of DNS reagent into an empty tube. To the other tubes, labelled 1g to 8g, add 3 ml of the appropriate glucose standard solution and 3 ml of DNS reagent. Place all the tubes in a boiling water bath for 5 minutes, then allow them to cool until still warm, and add 1 ml of Rochelle salt to each tube. Shake the tubes well. Add 3 ml of distilled water to each tube, shake, and cool to room temperature (Table 22).

Table 22: Preparation of glucose solutions for absorbance measurement

Test-tube	Blind g	1g	2g	3g	4g	5g	6g	7g	8g
Standard glucose solution (volumetric flask)	/	A	B	C	D	E	F	G	H
Standard glucose solution (ml)	0,0	3,0	3,0	3,0	3,0	3,0	3,0	3,0	3,0
DNS reagent (ml)	3,0	3,0	3,0	3,0	3,0	3,0	3,0	3,0	3,0
Distilled water (ml)	6,0	3,0	3,0	3,0	3,0	3,0	3,0	3,0	3,0
Rochelle salt solution (ml)	1,0	1,0	1,0	1,0	1,0	1,0	1,0	1,0	1,0

Preparation of fructose solutions for spectrophotometer measurement

Pour 3 ml of distilled water and 3 ml of DNS reagent into an empty tube. To the other tubes (labelled 1f to 8f) add 3 ml of the appropriate standard fructose solution and 3 ml of DNS reagent. Place all the tubes in a boiling water bath for 5 minutes, then allow them to cool until still warm, and add 1 ml of Rochelle salt to each tube. Shake the tubes well. Add 3 ml of distilled water to each tube, shake and cool to room temperature (Table 23).

Table 23: Preparation of fructose solutions for absorbance measurement

Test-tube	Blind g	1g	2g	3g	4g	5g	6g	7g	8g
Standard glucose solution (volumetric flask)	/	A	B	C	D	E	F	G	H
Standard glucose solution (ml)	0,0	3,0	3,0	3,0	3,0	3,0	3,0	3,0	3,0
DNS reagent (ml)	3,0	3,0	3,0	3,0	3,0	3,0	3,0	3,0	3,0
Distilled water (ml)	6,0	3,0	3,0	3,0	3,0	3,0	3,0	3,0	3,0
Rochelle salt solution (ml)	1,0	1,0	1,0	1,0	1,0	1,0	1,0	1,0	1,0

Measurement of absorbance (A) of glucose and fructose solutions

Measure the absorbance of each solution separately at 512 nm (A_{512}) using a spectrophotometer.

Then prepare the calibration curves: plot glucose-specific absorbance against glucose concentration, and fructose-specific absorbance against fructose concentration. Based on the results, draw calibration curves (Table 24 and Table 25).

Preparation of extract(s) from the plant material to be examined

Weigh 0.5 g of plant material (grass, onions, etc.) and crush in a mortar with distilled water. Filter the extract into a beaker and make up the filtrate to 50 ml with distilled water.

Measurement of absorbance of plant samples

Pipette 3 ml of the plant extract (sample) into a test tube and add 3 ml of DNS reagent. Heat the test tube in a boiling water bath for 5 minutes and then cool it under running water until it is still warm. Add 1 ml of Rochelle salt and shake well. Add 3 ml of distilled water and shake the tube. Cool the tube to room temperature, measure the absorbance at 512 nm (A_{512}), and record the results (see Table 26 and Table 27).

RESULTS AND OBSERVATIONS

Table 24: Absorbance (optical densities) for different glucose mass concentrations

Glucose concentration by mass γg (mg ml^{-1})	Absorbance (A)				Glucose specific A (Ā-A blank)
	1st measurement	2nd measurement	3rd measurement	Average absorbance (Ā)	

Table 25: Absorbance (optical densities) for different fructose mass concentrations

Fructose concentration by mass γg (mg ml^{-1})	Absorbance (A)				Fructose specific A (Ā-A blank)
	1st measurement	2nd measurement	3rd measurement	Average absorbance (Ā)	

Table 26: Absorbance (optical densities) for different glucose samples

Sample	Absorbance (A)				Glucose specific A (Ā-A blank)
	1st measurement	2nd measurement	3rd measurement	Average absorbance (Ā)	

Table 27: Absorbance (optical densities) for different fructose samples

Sample	Absorbance (A)				Fructose specific A (Ā-A blank)
	1st measurement	2nd measurement	3rd measurement	Average absorbance (Ā)	

Calculation:

Calculate the amount of reducing sugars present in the sample using the standard graph.

section 8

BIOTESTS FOR PLANT HORMONE-LIKE EFFECTS

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17. Exercise:

Triticum Test for the Determination of Cytokinin-like Effects

INTRODUCTION

Bioassays can be used to determine the presence of plant hormones or hormone-like substances. Plant hormones are natural plant growth regulators (PGRs). Both natural and synthetic PGRs, in small amounts, have a qualitative or quantitative effect on growth.

Plant hormones are a group of organic substances and are the most important internal regulators of growth and development. They are organic molecules present in plants at very low concentrations and do not serve as nutrients. They are synthesized in specific parts of the plant, transported through the plant, and trigger cellular responses in target cells or tissues (Vodnik, 2012).

High performance liquid chromatography (HPLC) and gas chromatography (GC) are the two main methods used to quantitatively measure the content of plant hormones, often followed by mass spectrometry (MS). Both methods are accurate but complex and expensive.

There are also classic methods for determining plant hormones and hormone-like substances, namely bioassays, which involve the use of plant material to detect the effect of plant hormone-like substances (Vodnik, 2012).

A bioassay is a testing system in which a specific response is detected and obtained by testing on living organisms or their parts. A bioassay is used to determine the presence or concentration of a specific chemical substance (Yopp, 1990).

Bioassays are based on the use of biological responses as a system for detecting biologically active substances such as plant hormones. In their simplest form, they are used to analyse the effect of a substance in comparison to a known concentration of the same substance.

One of the standardized bioassays for the determination of cytokinins is the wheat (*Triticum*) bioassay. It can be used to determine the cytokinin-like effects of 6-benzylaminopurine (BAP)-like substances. Cytokinins regulate the development of chloroplasts, along with other factors, light, nutrients, and developmental stage. If etiolated plants are treated with cytokinins before exposure to light, chloroplasts, chlorophylls, and photosynthetic proteins develop earlier in such plants than without the addition of cytokinins (Vodnik, 2012).

The wheat bioassay is based on cytokinin inhibition of chloroplast disintegration. Absorbance is measured using a spectrophotometer from chlorophyll extracts - segments of the first leaves of wheat exposed to different concentrations of the hormone BAP or extracts from the plants (samples) under investigation.

OBJECTIVES

- To determine the amount of cytokinins in a selected test plant using the *Triticum* bioassay.

TASK

1. Prepare wheat germ. Germinate the seeds in light for 7–10 days until the germ reaches a size of 10–13 cm.
2. Prepare known concentrations of cytokinin solutions for the calibration curve.
3. Determine the content of the cytokinin BAP-like hormone in the selected test plant.

MATERIAL

Scalpel, ruler, test tubes, test tube holder, rubber bands.

CHEMICALS

Cytokinin BAP (6-benzylaminopurine), wheat seeds for preparation of germ, plant material for examination.

PROCEDURE

Preparation of sprouts

Prepare the test material, germ, from wheat seeds. Soak the wheat seeds for 5 minutes in 2% sodium hypochlorite. Rinse them under running water for 2 hours.

Place the wheat seeds in a beaker that has been cleaned and disinfected with sodium hypochlorite or ethanol and prepare the water-soaked perlite. Disinfect your hands and plant the washed seeds 1 cm deep in the moist perlite or place the seeds on the perlite base provided and cover them with moist perlite to a height of 1 cm. Allow the seeds to germinate in the light for 1 week.

Preparation of aqueous extract from plant material

Prepare the extract of the examined plant by drying the weighed plant material in a lyophiliser at -50°C, and then crushing it to a powder using a mortar and pestle. Extract a known quantity of the crushed material in a known volume of water (50 or 100 ml), adding a few drops of alcohol to improve solubility and extraction.

Preparation of biotest

After one week, cut the wheat germ leaves 35 mm below the apical meristem into 10 mm long segments (see Figure 7). Carry out the bioassay of the sample (test plant) at the same time as the calibration curve test.

Prepare four parallel tests (4 tubes) for each known concentration of BAP (0, 0.3, 0.5, 1, 3, 10 mg l^{-1}) and three parallel tests for each extract of the test plant.

Pour 5 ml of the determined concentration and 5 ml of the test plant extract into a test tube and place ten 10–mm segments in each tube.

Cover the tubes to prevent evaporation and place them in complete darkness for 4 days.

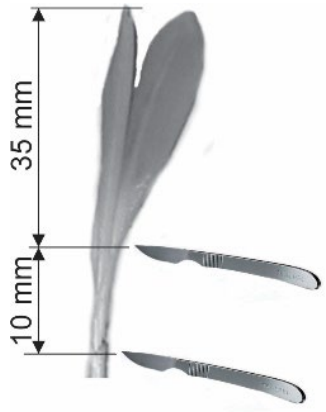

Figure 7: Preparation of segments from wheat sprouts for bioassay

Preparation of extracts for absorbance measurement

After four days, pipette or pour off the hormone solutions and extracts of the plant under investigation from tubes containing segments of wheat leaves. Add 8 ml of 80% ethanol to each tube containing the wheat leaf segments and cover to prevent evaporation. Heat the tubes in a water bath at 80–90°C.

After heating, make up the volume to the 10 ml mark with ethanol, and allow the tubes to cool to room temperature. Begin by measuring the absorbance at 645 nm. Record the absorbance measurements for the known BAP concentrations and samples in a table (Table 28 and Table 29) and calculate the average.

Using the measured absorbance values for the known BAP concentrations, draw a calibration curve and determine (calculate) the concentration of hormone-like substances in the samples (Table 28 and Table 29).

RESULTS AND OBSERVATIONS

Table 28: Measurement of absorbances (optical densities) for known BAP concentrations

Hormone BAP concentration (mg l^{-1})	Absorbance (A)			Average absorbance ($\bar{A}$)
	1st measure	2nd measure	3rd measure	

Table 29: Measurement of absorbances (optical densities) of samples

Sample	Absorbance (A)			Average absorbance ($\bar{A}$)
	1st measure	2nd measure	3rd measure	

NOTES

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18. Exercise:

Mungo Test for the Determination of Auxin-Like Effects

INTRODUCTION

The mungo test is based on the rooting of the germ of the mung bean (Vigna radiata). It is a relatively simple bioassay (Hess, 1961), does not require expensive equipment and is relatively insensitive to the presence of inhibitors. See also the introduction to Exercise 17.

OBJECTIVES

– To determine the amount of auxins in a selected test plant using the Mungo (Vigna) bioassay.

TASK

1. Prepare the germ of the mung bean. Germinate the seeds in light for 7–10 days until the germs reach a size of 10–13 cm.
2. Prepare known concentrations of auxin solutions for the calibration curve.
3. Determine the auxin IBA-like hormone effects of the selected plant studied.

MATERIAL

Growth chamber or greenhouse, light source, plastic trays, glass vials (25 mm × 90 mm).

CHEMICALS

Sterile perlite or vermiculite, sodium hypochlorite solution (NaOCl, 0.33 %), auxin IBA (indole-butanoic acid), mung bean seeds, plant material for the study.

PROCEDURE

Preparation of Sprouts

Soak the seeds of mung beans (Vigna radiata) in a 0.33% sodium hypochlorite solution for 4 minutes and, after sterilization, rinse them overnight under a small stream of running water. Plant the imbibed seeds in moistened perlite (vermite or vermiculite) in plastic tubs. Transfer the trays to the light in a growth chamber, or to a bright and warm place, until they reach 10–13 cm in size. Germination is ready for trials after 7 to 10 days.

When the seedlings are of a suitable size, gently pull them out of the substrate and cut the stem and roots of each one separately with a clean scalpel or razor blade, at about 3 cm below the cotyledons (Figure 8).

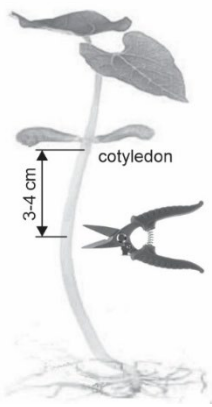

Figure 8: Preparation of bean sprouts for bioassay

Preparation of an aqueous extract of the plant material to be examined

Prepare the extract of the test plant by drying the weighed plant material in a lyophiliser at -50°C, then crushing it to a powder using a mortar and pestle. Extract a known quantity of the crushed material in a known volume of water (50 or 100 ml), adding a few drops of alcohol to improve solubility and extraction.

Preparation of the biotest

Prepare an appropriate volume of different concentrations of IBA growth regulator. Prepare the following concentrations of IBA: (0, 0.3, 0.5, 1, 3, 10 mg l^{-1}). Fill four low tubes (5–10 cm in height) with 10 ml of each IBA concentration.

Record on each tube the exact date of the start of the bioassay and the hormone concentration or type of plant extract (sample) to be tested. Place four bean sprouts in each tube. Leave the bean sprouts in the individual solutions of different IBA concentrations for approximately 24 hours. After 24 hours, replace the IBA solutions with 10 ml of distilled water. The bioassay lasts 7 days. During this period, add the water to the tubes as needed. After 7 days, terminate the bioassay. Remove sprouts from the experimental solutions and count the roots formed that are longer than 1 mm. Record the results in an Excel spreadsheet.

The number of roots is directly proportional to the auxin concentration within the test area.

For each concentration, calculate the mean number of roots, the standard deviation (SD), and the standard error (SE).

From the mean root numbers obtained for all known IBA concentrations, prepare a calibration curve (graph) to determine the IBA or other auxin effects of your test plants (samples).

Conduct the sample bioassay at the same time as the calibration curve test. Prepare the sample bioassay following the same procedure as the calibration curve. The only difference is that, instead of adding a known concentration of aqueous auxin solution to the tube of bean plants, you add a known amount of aqueous extract of the plant sample for which you are interested in determining the plant hormone-auxin effects. After seven days, remove the sprouts from the experimental solutions and count the roots formed that are longer than 1 mm. Calculate the mean number of roots, SD and SE. From the previously prepared calibration curve, determine the effects of auxin-like substances in your samples.

RESULTS AND OBSERVATIONS

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References

Ambrožič-Dolinšek J., Ciringer T. 2022. Fiziologija rastlin: Priročnik z navodili za vaje. Univerzitetna založba Univerze v Mariboru. https://doi.org/10.18690/um.fnm.3.2022

Bidwell R. G. S., 1974. Plant physiology. Macmillan Publishing, NY.

Gabrovšek K., Gogala N. 1991. Navodila za vaje iz fiziologije rastlin, Oddelek za biologijo, Biotehniška fakulteta, Ljubljana.

Gros N., Harrison T., Štrumbelj Drusany I., Kapun Dolinar A. 2010. Science In School. Prevzeto 30. marec 2011 iz Spektrometrija v šoli: eksperimenti z izkustvenim pristopom: http://www.scienceinschool.org/2010/issue14/spectrometer/slovene

Greulach V. A., 1973. Plant physiology. Macmillan Publishing, NY.

Hess C. E., 1961. The mung bean bioassay for detection of root promoting substances. Plant Physiology 36(I): Suppl. 21.

Kühnle J. A., Fuller G., Corse J. and Mackey B. E. 1977. Antisenescent activity of natural cytokinins. Physiol. Plant., 41: 14–21.

Krajnčič B. 1993. Fiziologija in biokemija rastlinskih hormonov. Fakulteta za kmetijstvo, Maribor.

Kutschera U. 1998. Grundpraktikum zur Pflantzenphysiologie. Quelle & Meyer Verlag Wiesbaden.

Likar M., Regvar M. 2003. Praktikum fiziologije rastlin. Scripta, Študentska založba, Ljubljana.

Likar M., Vogel-Mikuš K. 2013. Fiziologija rastlin: navodila za laboratorijske vaje [S. l.: s. n.].

Mohr H., Schopfer P.1995. Plant physiology. Springer-Verlag.

Sadasivam S., Manickam A. 2007. Biochemical Methods. Prevzeto 30. marec 2011 iz New Age International: http://www.newagepublishers.com/samplechapter/000091.pdf

Salisbury F.B., Ross C.W. 1991. Plant Physiology. Wadsworth Publishing Company Belmont, CA.

Sitte P., Weiler E.W., Kadereit J.W., Bresinsky A., Körner C. 2002. Lehrbuch der Boranik für Hochsculen. Begründet von Strasburger E., Noll F., Schenck H., Schimper. Spectrum Akademischer Verlag Heidelberg, Berlin.

Stušek P., Podobnik A., Gogala N. 1997. Celica. DZS Ljubljana.

Šarič M. R., Fiziologija biljaka. 1974. Naučna knjiga, Belgrade.

Taiz L., Zeiger E., Moller IM., Murphy A. 2018. Plant Physiology and Development. Sinauer Associates, Oxford University Press, NY.

Yopp J. H. 1990. Bioassays for plant hormones and other naturally occurring plant growth regulators. In: Mandava, N. B. (ed.): CRC Handbook of Natural Pesticides: Methods. Vol. 1. Theory, Practice, and Detection. CRC Press (Boca Raton, Ann Arbor, Boston), 329–477.

Vinković Vrček, Loretić. 2010. Aditivi u hrani. Vodič kroz E-brojeve. Zagreb. Školska knjiga.

Vodnik, D. 2012. Osnove fiziologije rastlin. Ljubljana: Oddelek za agronomijo,
Biotehniška fakultete.

APPENDIX

Appendix 1: Spectrophotometry

Absorption spectrophotometry is a method for measuring the amount of light absorbed by a sample at a given wavelength. This method also allows the concentration of a substance in a sample contained in a cuvette of a given length (l) to be determined by comparison with a standard. The most useful measurement of light is absorbance (A), also called optical density. It is defined as $A = \log I_0 / I$, where I_0 is the intensity of light incident on the sample, and I is the intensity of transmitted light.

The absorption of light is represented graphically by relating the wavelength (l) to the abscissa (the independent variable) and the absorbance (A) to the ordinate (the dependent variable). The resulting graph is called an absorption spectrum and shows the wavelengths most strongly absorbed by the sample (in this case, the dye).

Absorbance (optical density) is measured with a spectrophotometer, and the method is called absorption spectrophotometry. The operation of a spectrophotometer is shown in the figure below.

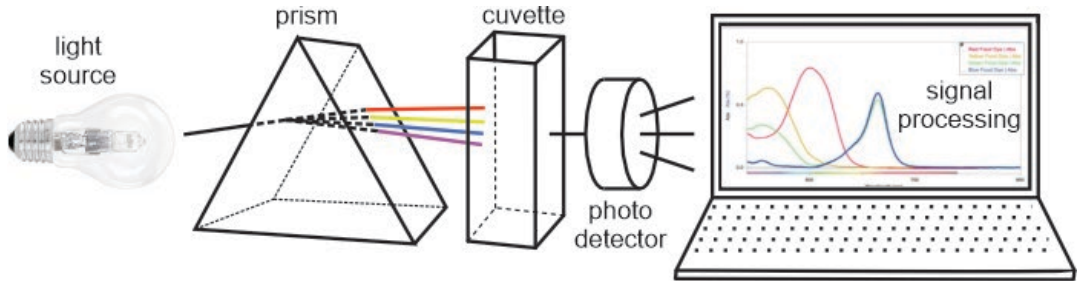

Figure 9: Schematic diagram of the spectrophotometer operation

The device consists of a light source, a monochromator with a mechanism (which can be a prism) to select the wavelength, a sample holder with a glass cuvette, a photodetector, and a recorder or computer. The wavelength of the light varies by rotating the prism in the monochromator. The light passes through the monochromator, which splits the polychromatic light into several wavelengths, of which only one monochromatic wavelength passes through the sample in the cuvette to the photodetector.

The optical density (A, absorbance) of the sample is related to the concentration by Beer's law:

$$\mathbf{A = \varepsilon \cdot c \cdot l} \qquad (6)$$

Here c is the concentration, usually measured in moles per liter, l is the length of the light path (cuvette), usually 1 cm, and ε is a constant called the molar extinction coefficient with the unit liter per mole per centimetre (l mol-1 cm^{-1}). The usual ε for chlorophyll is 100,000 l mol^{-1} cm^{-1}.

Appendix 2: Selected basics of chemical stoichiometry

Percent concentration

We distinguish between mass and volume percentages. If the text does not specify which, the percentages by mass are meant.

The concentration in mass percentages is expressed as grams of solute per 100 g of solution.

The concentration in volume percentages is expressed in parts by volume of the substance in 100 parts by volume of the solution.

Molar concentration

The concentration is expressed as the number of moles of solute in 1000 ml (1 liter) of solution (mol l^{-1}). The molecular mass in grams (g) is calculated from the relative molecular masses, Mr, of the individual elements. These values are found in handbooks and are also written on the packaging of all originally packaged chemicals. Other symbols used to express molarity include (mol l^{-1} = M) and millimolarity (mmol l^{-1} = mM) and micromolarity (µmol l^{-1} = µM).

Mass concentration

Concentration is expressed as the mass of a solute in a given volume of solution, such as milligrams per liter (mg l^{-1}).

Unit conversions:

1 mm^3 = 1 µl = 1000 nl = 1 x 10^{-6} pl
1 cm^3 = 1 ml = 10^{-3} µl = 1000 µl
1 μm^3 = 10^{-9} mm^3 = 10^{-9}µl = 10^{-6} nl = 10^{-3} pl

Plant Physiology: Manual For Laboratory Exercises

Jana Ambrožič-Dolinšek, Terezija Ciringer
University of Maribor, Faculty of Natural Sciences and Mathematics, Maribor, Slovenia
jana.ambrozic@um.si, terezija.ciringer@um.si

This handbook is intended for students in biology and ecology programs (including conservation), as well as future biology teachers who wish to become familiar with experimental work in plant physiology. It contains eight sections covering plant pigments, plant respiration and photosynthesis, plant enzyme activity, water regulation in plants, plasmolysis, plant mineral nutrition with identification of symptoms of individual nutrient deficiencies, methods for determining sugar content, and bioassays for plant hormones. Each exercise begins with an introduction, followed by the objectives, tasks, materials, and procedure for conducting the exercise. The exercises are supported by images of the experiments, tables, chemical formulas for calculating specific physiological parameters, and instructions for working with chemicals and equipment.

DOI
https://doi.org/10.18690/um.fnm3.2026

ISBN
978-961-299-156-2

Keywords:
experimental exercises, textbook, plant physiology, plant biology, laboratory equipment and procedures, laboratory plants

University of Maribor Press

University of Maribor

Faculty of Natural Sciences and Mathematics