PEOPLE'S DEMOCRATIC REPUBLIC OF ALGERIA
MINISTRY OF HIGHER EDUCATION AND SCIENTIFIC RESEARCH
FERHAT ABBAS UNIVERSITY, SETIF 1
FACULTY OF NATURAL AND LIFE SCIENCES
DEPARTMENT OF BASIC STUDIES

**Polycopid of Practical Works**

# « Plant physiology »

Designed For second year students (LMD)
FIELD: Natural and Life Sciences (NLS)
SECTOR: Ecology and Environment

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Academic year 2024-2025

Modul: Plant physiology

Semester: 3rd Semester

Teaching Unit: Discovery

Code: UED 2.1.1

Credits: 2

Coefficients: 2

**Recommended background knowledge:** This practical course consists of a series of laboratory experiment (Worksheets) to familiarize students with main concepts and techniques in plant physiology. The practical course is intended to complement the lecture course, which is an absolute pre-requisite.

**Methodology:** The methodology of teaching Plant Physiology is based on State Educational Canvas (Educational program) adopted by the Ministry of Higher Education and Scientific Research.

**To understanding** the questions that Physiologist's study, the methods they use, and the questions that remain unanswered about how plants grow, develop and sense their environment, and to acquire a broad knowledge of common methods and techniques used in plant physiology, **we utilize** for Each chapter a process (worksheet) that involve students to observed, measured, classified, experimented, calculates, plots and analyzes several parameters and criteria based on equations and diagrams. These worksheets assist students in understanding, discussing, inferring, predicting and interpreting data to **mastering** the concept of how to "scientifically" investigate plant phenomenon.

## Practical Works Description:

The practical course consists of **two hours** laboratory biweekly (every two weeks). The labs are intended to help students visualize main basic concepts and common techniques in plant physiology. Students will learn basic data analysis techniques and how to interpret results from simple experiments. At the end of each laboratory students are asked to answer the questions posted in the worksheets and to write a short scientific report with an introduction, methods, results and interpretation.

Although students work in pairs in the classroom and are encouraged to discuss the results in groups, each student is required to write her/his own reports independently. Therefore, different wording and writing structure is expected.

## Reports:

All reports are due at the laboratory session following the one in which the experiment was completed. The scientific reports must follow the following format. It should have the following five main sections clearly labeled: Cover page, Introduction, Materials and methods, Results and Discussion.

**Title** should be as short but as informative as possible.

**Introduction** should provide some conceptual background and include a general description of the problem or topic being studied. Also make sure to identify the objectives or purposes of the experiment.

**Materials and Methods** of a scientific paper is a written description of the experimental procedures, usually describe the methods in enough detail to allow someone else to duplicate the experiment.

**Results s**ection should contain a brief description of the findings use the past tense. This section should include tables or figures of the elaborated data attached at the end of the paper.

The text should briefly describe the information in the tables or figures. If more than one table or figure are necessary, number them sequentially (e.g. Table 1, Table 2 etc., or Figure 1, Figure 2, etc. A graph is referred to in the text as a figure (not graph, chart or any other term).

Generally, results of an experiment are presented in the form of a table or graph. The choice depends on the actual experiment and the nature of findings. Data presented as graph are quickly assimilated and sometimes need less space. Table should be brief and complete with all the relevant information. Each table and graph should have a title and required details so that they can be easily understood without reference to written report. Results in tables and figures should be presented clearly. A table legend appears above the table and a figure legend appears below.

The units of expression of results should be given at the top of each column of figures and not repeated on each line of table. The unit should be adjusted in such a way that the figures are convenient to understand.

If results are presented as graph, it should be neatly drawn and symbols of uniform size be used. All results should have extent of experimental error (as critical difference or standard deviation). If the curve needs to be extrapolated it should be shown by dotted lines.

**Discussion section** should include a summary of the principal findings together with an explanation or interpretation. What conclusions can draw with regard to the objectives described in the Introduction.

**Conclusions and Discussions**

The reporting of a result must always be followed by a conclusion which proves the validity of the experiment. The conclusion should be brief and accurate without repetition of ideas. If the results

obtained were not as expected, the possible reasons should be suggested or pointed out. A brief discussion of the findings should also be included narrating the reasons in support of the findings.

**Practical Works Program:** A core of 10 laboratory experiments was designed to complement the concepts and processes introduced in lectures. The objectives of the core labs are to(i) provide structured, hands-on experience with the study of plant physiology, (ii) teach the principles and skills used in modern techniques of measurement.

# Contents :

**GENERAL GUIDELINES FOR EXPERIMENTATION AND PLANT ANALYSIS**

## Introduction

To make laboratory work more meaningful it is advisable to follow certain basic guidelines. Before starting an experiment read carefully the protocol, the assigned objective and the hypothesis involved. Then prepare the plan of work before actually starting the experiment. Do not be a "filter paper scientist". Record all primary operations and data directly in your bound notebook. Data recorded in discs of filter paper on loose sheets of paper are likely to get lost. Data should be recorded in tabular form in well-defined format. All the data obtained should be recorded in your report no matter how unsatisfactory the data may appear to be. Do not feel perturbed if data, negative to your expectation, are obtained. Negative data are more meaningful and pave the way for exploration of protocol and testing of other components. It also provides opportunity to indicate the loopholes in your experimentation.

Graphical presentation of data should be done in order to quicker understanding of the experimental results. Finally, a concise report should be presented stating the methodology, results obtained and a brief discussion about the findings. At the end of day, your working bench should be as clean as it was in the beginning of the experimental operations.

## Control of accuracy

Accuracy may be defined as the degree of conformity to the truth. A good researcher/student needs to obtain very accurate measurements at the working bench. He is also required to present an accurate account of the experiment. Before actual experimental work a precise plan of work is mandatory. In addition, the protocol for experiment should precisely be followed for accurate results. Problems faced during work, arising due to the variations in plant materials, researchers, working conditions, etc., should clearly be stated in the report so that those who read it should have prior information about the difficulties which they can encounter during work.

## Measurements and errors

Laboratory work always involves certain measurements which are liable to errors. The potential source of these errors should always be appreciated. Errors may be random, or caused due to carelessness or inaccurate instruments or impure chemicals. Errors can also arise from careless experimental work such as using apparatus wrongly, preparing and measuring solution inaccurately.

Even modern instruments can produce strange results if the operational instructions are not followed correctly or the optimum conditions for the instrument is not met. Such errors can be rectified and with good practice can be eliminated.

An additional factor to be considered when working with living material is the biological variations. For biochemical measurements, any single value cannot be considered as correct but it has a range of so-called normal values. For example, if a plant material is free from stress and healthy then any biochemical value may fall within the normal range.

# PW N°1: DETERMINATION OF WATER CONTENT (PWC) IN PLANT TISSUES.

## I. INTRODUCTION:

Several variables allow to characterize the water state of the plant; These quantities are based on the measurement of the amount of water contained in a sample. We mainly distinguish:

**Moisture Content % (MC):** Plant weight is measured as fresh weight (the total weight including water) and dry weight (the weight after all water is removed), and these are used to calculate moisture content.

**MC (%)** = [(Fresh Weight - Dry Weight) / Dry Weight] x 100= (FW− DW/ DW) × 100

The amount of water contained is given by the weight difference between fresh matter and dry matter.

Water Weight =**WW**= (FW− DW)

Standard method for determining dry weight by drying a sample in an oven at 70-80 °C. This process is repeated until the weight becomes constant, this constant weight, or "dry weight," represents the sample's mass after all the water has evaporated.

**Plant Water Content (PWC)**

Plant water content **(PWC)** typically refers to the total amount of water present in a plant tissue, expressed as a percentage of water in the leaf relative to its fresh weight (FW) of that tissue.

The Plant Water Content (PWC) is therefore the ratio between the weight of water in a sample (WW) and its fresh weight (FW). It can be calculated using the formula:

Plant Water Content (**PWC)**= (WW/ FW) × 100= (FW− DW/ FW) × 100

## II. MATERIAL REQUIRED:

- Freshly leaf samples from different plants.
- Scissors
- Electronic weight balance
- Petri dish (Glass)
- Drying oven (70°C).
- Timer

## III. PROCEDURE:

1. Place small leaf fragments of different species in glass petri dishes.
2. Obtain the fresh weight (FW) of the leaf samples.

3. Place the leaf samples in a labeled Petri dish and dry them in a Drying oven at 70- 80°C for 10min.

4. Record the initial time since start of experiment (T0 = 0min)
5. Reweigh the leaf samples after taking them out of the oven.
6. Mark or record the weights of the samples every 10 minutes (T1, T2, T3…) until the material keeps a constant weight (DW; dry weight).
7. Note down in the results table (Table 1)

| Time (min) | 0 (FW) | 10 | 20 | 30 | 40 | 50 |
|---|---|---|---|---|---|---|
| Weight of plant 1 | | | | | | |
| Weight of plant 2 | | | | | | |
| Weight of plant 3 | | | | | | |

## IV- Analysing the results

- For each species, (1), (2) and (3) using the equations above:
- Calculate the Water Weight (WW) and the Moisture Content % (MC).
- Calculate the Plant water content (PWC).
- Record the result in your table (Table 2).

**Tableau 2**. Water Weight (WW), Moisture Content % (MC) and Plant water content (PWC) for different species studied.

| **Species** | **Fresh Weight (g)** | **Dry Weight (g)** | **Water Weight (g)** | **Moisture Content %** | **Plant water content %** |
|---|---|---|---|---|---|
| 1 | | | | | |
| 2 | | | | | |
| 3 | | | | | |

- Comment and interpret the obtained results.
- Compare the results obtained between species (1), (2) and (3). Explain.

For the report of the practical work, it is recommended to follow the following plan:

**The recommended outline of the report:**

- Cover page
- Aims
- Material required
- Procedure
- Results and interpretation
- Conclusion

**Importance:** PWC is crucial for understanding the hydration status of the entire plant, which affects physiological processes such as photosynthesis, nutrient transport, and overall growth.

PWC provides insights into the turgor pressure and water availability in leaves, which is essential for maintaining cell structure and function. It's a good indicator of plant stress and water availability.

Need to Calculate PWC

1. Water Status Assessment: Both metrics help assess the water status of plants, but from different perspectives (whole plant vs. specific leaves).
2. Stress Evaluation: They are useful in evaluating plant responses to environmental stresses such as drought, salinity, and temperature extremes.
3. Physiological Studies: Understanding how different parts of the plant manage water can inform studies on growth, development, and adaptation strategies.
4. Agricultural Practices: These measures can guide irrigation strategies and improve crop management by providing insights into plant health and water needs.

In summary, while both metrics are related to water content, they serve different purposes in plant physiology and ecology, helping researchers and farmers alike to understand and manage plant health effectively.

# PW N°2: DETERMINATION OF STOMATAL DENSITY AND DISTRIBUTION ON LEAVES.

**AIMS**:

- To study the pattern and distribution of stomata in both the upper and lower leaf surfaces.
- Differentiate between dicot and monocot stomata.

Stomata are minute pores found on the epidermis of leaves and young shoots of plants that are used to control exchange of gases. The pore is surrounded by a pair of specialized cells called the guard cells that are responsible in regulating the size of the opening.

Water is released through the stomata into the atmosphere in the form of water vapor through the process called transpiration. Besides this, the exchange of oxygen and carbon dioxide in the leaf also occurs through the stomata.

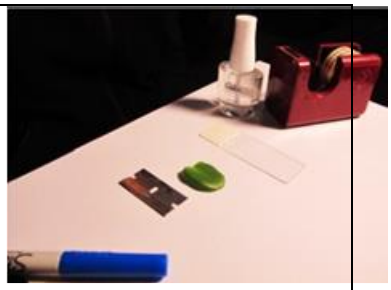
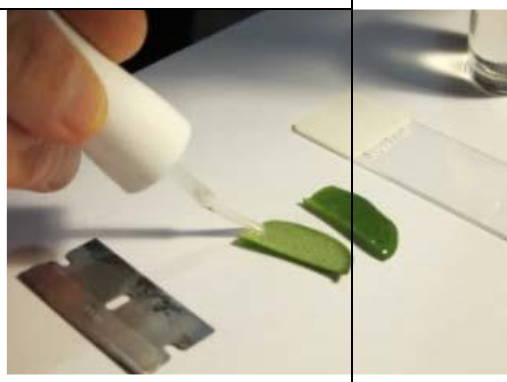
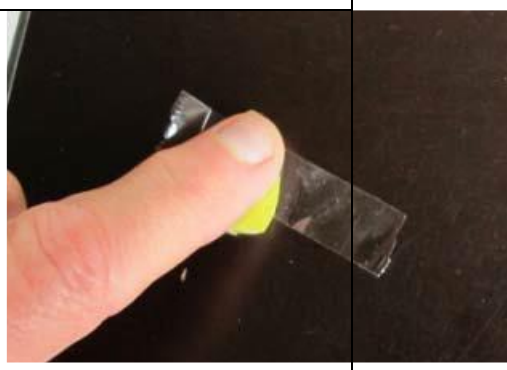
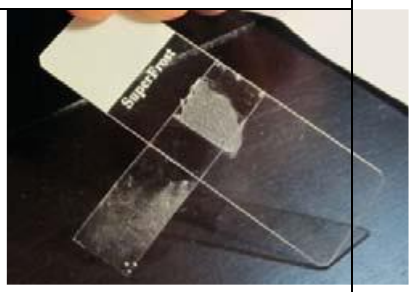
The distribution of stomata on the upper and lower surfaces of the leaf can be studied by removing the peels of the leaf from the upper and lower surfaces and observing the same under a microscope.

## II. MATERIALS:

- Plants with suitable leaves. Smooth leaves without many leaf hairs are usually best.
- Clear nail polish.
- Clear sticky tape
- Forceps.
- scalpel or razor blade.
- Compound microscope.
- Microscope slides.
- Marker pen.
- Digital camera to capture microscope images of the leaf surfaces – this greatly aids in counting stomata and is a record of the results for future reference.

## III. PROCEDURE:

This method uses clear nail polish to make an impression or cast of the leaf surface. The cast is removed with sticky tape and placed on a microscope slide.

| | |
|---|---|
| 1. Collect materials. Find a suitable leaf. Identify the upper and lower surfaces of the leaf. |  |
| 2. Spread a thin layer of clear nail polish on EACH SURFACE (upper side and lower side)<br>3. Wait for the nail varnish to dry (approx. 5 minutes). |  |
| 4. Place a strip of clear stick tape over the nail polish |  |
| 5. Peel off the sticky tape; the layer of nail polish should come off with the tape. |  |
| 6. Place the tape with leaf impression on a microscope slide.<br>7. Place the impression from the other side of the leaf on the other part of the slide.<br>8. Label the slide with the plant name, and the upper and lower surface.<br>9. Repeat the process with peels removed from a monocot leaf. Record your observations. |  |

**Observations and recording results**

a) Make sure that you can identify the stomata compared to epidermal cells.

b) Choose a magnification that gives a number of stomata you can keep track of when counting.

c) Decide on rules for counting (e.g., the whole stoma must be in view to be counted).

d) Count the stomata in at least 3 FOVs (field of views) on each leaf surface.

e) Record the results in a table and calculate the average number of stomata in the field of the microscope.

**IV- Results**

Example results table

| Plant Name | Magnification | Surface (upper/lower) | FOV # | Number of stomata in entire FOV | average number of stomata | *Stomatal density stomata/mm2 |
|---|---|---|---|---|---|---|
| 1……. | **40x / 100x / 400x** | upper | **1** | | | |
| | | | **2** | | | |
| | | | **3** | | | |
| | **40x / 100x / 400x** | lower | **1** | | | |
| | | | **2** | | | |
| | | | **3** | | | |

* Using previous microscope calibration values. Field of view is how much of your specimen or object you will be able to see through the microscope.

| **Objective magnification** | **Ocular eyepiece** | **Total magnification** | **Diameter of the Field of view FOV** |
|---|---|---|---|
| 4x | 10x | 40x | 4.48 mm (is around 4480μm). |
| 10x | 10x | 100x | 1.8 mm (is around 1800 μm). |
| 40x | 10x | 400x | 0.46 mm (is around 450 μm). |

Area of FOV = $\pi r^2$ (r is the radius of the view field).

Stomatal density = number of stomata in entire FOV / area ($mm^2$)

- Repeat the procedure with different leaves
- Draw the diagram of a stoma and label its parts.
- Compare stomatal density on the upper and lower epidermis of these plants
- Compare the distribution of stomata on the two epidermises of dicot and monocot leaves

**PRECAUTIONS**

**.** Peel should be taken from freshly plucked leaf.

**.** Peel should not be allowed to dry.

**.** Leaf peel should not be over stained.

**.** The slide should not be dirty.

**.** Peel should be placed in centre of slide.

**.** Curling of peel should be avoided while placing it on slide.

**.** The epidermal peel should be small in size.

## Measurements to take

Use a stage micrometer to measure the diameter of the field of view

This has to be at the same magnification power that you used when counting the stomata

A typical microscope allows the scientist to look at a field of view of about 0.5 mm diameter when on full power (× 400)

You will have calculated a mean number of stomata per field of view from the previous stage

Worked example

A study reveals a mean count of 16 stomata per field of view at a magnification of × 400.

The stage micrometer calculates the diameter of the field of view at a magnification of × 400 to be 0.46mm

Calculate the stomatal density based on these data. Give units in stomata per $mm^2$

Use a value of $\pi = 3.14$ and give your answer to the nearest whole number of stomata.

## Answer:

Step 1: Calculate the radius of the field of view

$$\text{Radius} = \text{Diameter} \div 2$$

$$\text{Radius} = 0.46 \text{ mm} \div 2 = 0.23 \text{ mm}$$

Step 2: Calculate the area of the field of view

$$\text{Area} = \pi r^2 = \pi \times 0.23^2$$

$$\textbf{Area} = \mathbf{0.1662 \text{ mm}^2}$$

Step 3: Divide the mean number of stomata by the area of the field of view to calculate density

$$\text{Density} = 16 \div 0.1662 = 96.27 \text{ stomata per mm}^2$$

Step 4: Round to the required precision (nearest whole number)

$$\text{Density} = 96 \text{ stomata per mm}^2$$

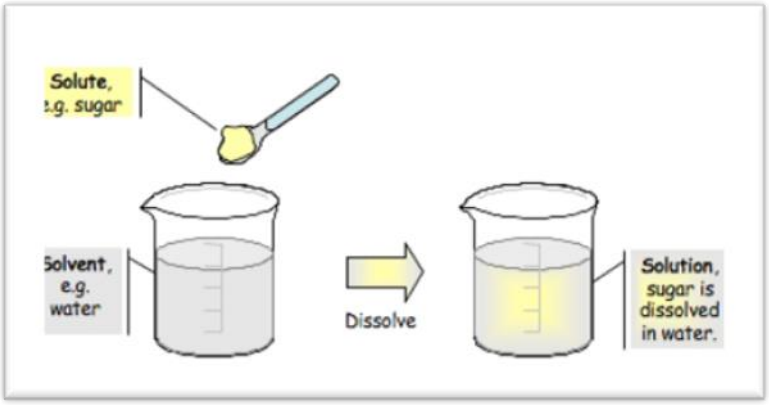
# PW N°3: Preparing chemical solutions

## A- Basic Concepts of Preparing Solutions

### A-1- What is SOLUTIONS?

A solution is a homogeneous mixture of one or more solute(s) dissolved in a solvent. A solute is a substance that is dissolved in a liquid solvent to produce a solution.

**Exemple :**




### A-2-Concentration of a solution

The concentration of a solution refers to the quantity of solute dissolved in a particular quantity of solvent or solution. There are several ways of expressing solution concentrations such as :

1. Concentration in moles per litre, molar concentration or molarity (mol/L or mol L-1 or M)
2. Concentration by percentage (either %w/v or % v/v or sometimes %w/w)
3. Concentration in grams per litre (g/L or g L-1)
4. Preparing solutions by dilution

### A-2-1 - Concentration in grams per litre (g/l)

A solution can be prepared by dissolving a known mass or volume of solute in a known amount of solvent. Concentration is expressed as grams of solute dissolved in one litre of solution.

Example:

Calculation for preparing 300 mL of a sucrose solution at a concentration of 5 g/L.

As only 300 mL (0.3 L) of solution is required, only a fraction of the 5 g will be needed.

To find the quantity of sucrose required, the concentration is multiplied by the fraction of litres required:

$$m = 5\ g/L\ x\ 0.3\ L$$

$$m = 1.5g$$

**A-2-2 Concentration in moles per litre, molar concentration or molarity CM**

In chemistry, molarity is the most frequently used method of expressing concentration of a solution. **Molarity** indicates the number of moles of solute dissolved in a litre of a solution; has the symbol **M**, and the unit, *moles per litre (mol/L).*

The concentration of a solution in mol/L can be calculated using the formula

$$\mathbf{c(M)= n\ /}\ \mathrm{V}\ (moles\ per\ litre)$$

$$Concentration = c = number\ of\ moles\ of\ solute\ (mol)\ /\ volume\ (L)$$

Where *c* = concentration of the solution in moles per litre (mol/L)

*n* = number of moles of solute (mol)

V = volume of solution in litres (L)

To find the number of moles of solute in a certain volume of a solution of known concentration

The equation can be rearranged

$$n = c\ x\ V$$

## A molar solution

The symbol M is pronounced 'molar'. Molar solutions use the molecular weight of a solute to calculate molar concentration in a litre of solution.

The molecular weight can be found on the chemical bottle label, in a data book or safety data sheet (SDS), or by adding together the atomic weights of all of the atoms, which appear in the chemical formula of the substance.

e.g. NaCl

1 sodium atom 1 x 22.9 g = 22.99 g

1 chloride atom 1 x 35.45 g = 35.45 g

Molecular weight = 58.44 g

Therefore, a 1M solution of sodium chloride consists of 58.44 g of NaCl dissolved in 1 L of water.

## B- Method to prepare a solution using a solid chemical in 1L of distilled water

1. Weigh the number of chemicals using an electronic balance, dry watch glass or weighing boat.

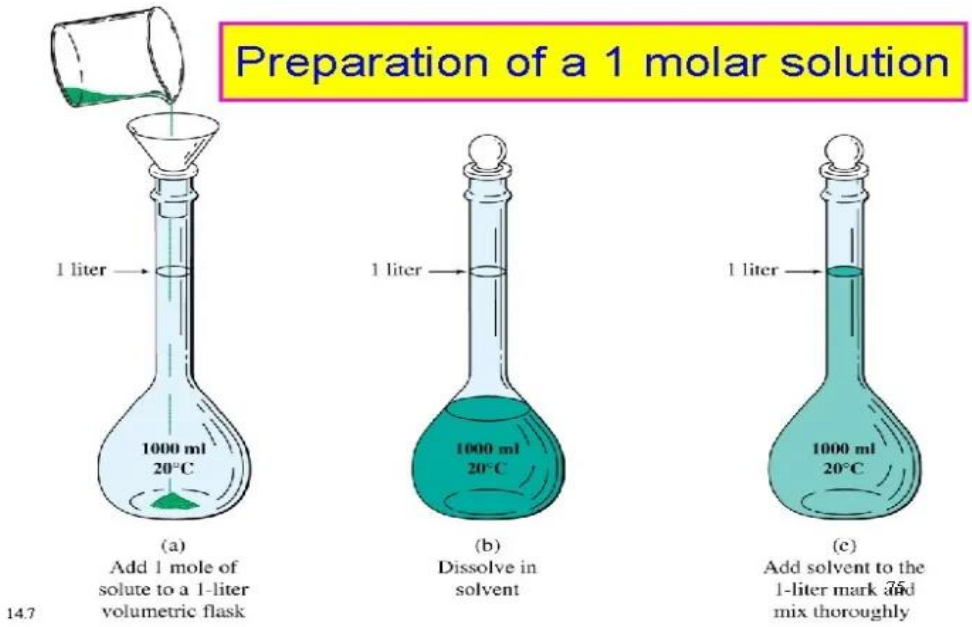
3. Carefully transfer the weighed chemical to a beaker or volumetric flask containing about two thirds of the final solution volume of distilled.

4. Stir to dissolve with a stirring rod or on a magnetic stirring platform.

5. The solution should be mixed well to obtain a homogeneous solution

6. Once dissolved transfer the solution to a 1 L measuring cylinder or volumetric flask.

7. Make up to 1 L with distilled or deionised water



# PW N°4: Osmosis in Plant Tissue.

**AIMS**:

- Recognize a solution and its composition of a solute and solvent.
- To demonstrate the process of osmosis with varying solution concentration
- Define the process of osmosis in tissues.
- Estimation of osmolarity in tissues by bathing samples in different solutions.
- Differentiate and identify isotonic, hypertonic, and hypotonic solutions.

**Osmosis** is the diffusion of water molecules, from a region of high-water concentration, to a region of lower water concentration, across a partially permeable membrane"**.**

In this worksheet, you will learn how to investigate the effect that a range of **salt concentrations** has on the mass of plant tissue (in this case the potato, but other root vegetables would also do the job). By creating potato strips of similar size and placing them in different concentrations of salt solution, the changes in their mass can be measured as they lose or gain water by osmosis. This experiment would also work with different concentrations of sugar solution. You will also discover how to work out the exact concentration of the cells of the potato you use in the experiment.

## II. MATERIAL REQUIRED :

- Potato tubes - 6 Test tubes + rack -Wash bottles
- Magnetic stirrers - knives - Pipette
-Erlenmeyer Flask (250 ml) - Weight balance
- Distilled water - Sugar or sodium chloride (NaCL) solution

## III. PROTOCOLE EXPERIMENTAL

- **Preparing chemical solutions**

### 1- Preparing standard solution

Prepare 1 molar (**M**) solution of sodium chloride (salt).

The symbol **M** is pronounced 'molar'. Molar solutions use the molecular weight of a solute to calculate molar concentration in a litre of solution.

e.g., **NaCl**

1 sodium (Na) atom 1 x 22.9 g = 22.99 g

1 chloride (Cl) atom 1 x 35.45 g = 35.45 g

Molecular weight = 58.44 g

Therefore, 1M solution of sodium chloride consists of 58.44 g of NaCl dissolved in 1 L of water (solution).

**2- Preparing serial dilution salt concentrations**

- Take 6 Test tubes and label them 1,2,3,4, 5 and 6.
- Prepare a range of 6 different salt concentrations containing 0M, 0.2M, 0.4M, 0.6M, 0.8M and 1M in each. Each Test tube had 10 milliliters of solution total (Table 1).

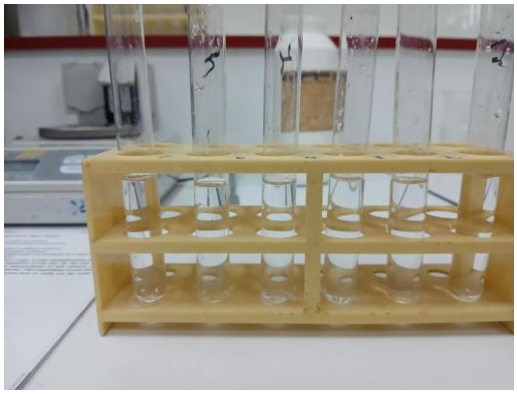
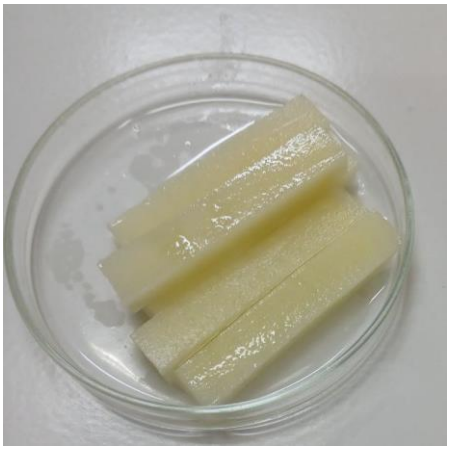


**Table 1:** Serial dilution table for preparation of final salt (NaCL) concentrations.

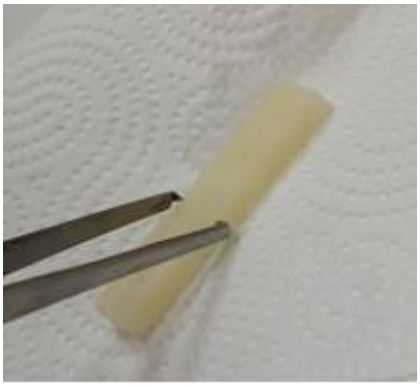
| **Tubes N°** | **1** | **2** | **3** | **4** | **5** | **6** |
|---|---|---|---|---|---|---|
| **Concentrations molaires** | 0 M | 0.2 M | 0.4 M | 0.6 M | 0.8 M | 1 M |
| **Standard solution** | 0 ml | 2 ml | 4 ml | 6 ml | 8 ml | 10 ml |
| **Distilled water** | 10 ml | 8 ml | 6 ml | 4 ml | 2 ml | 0 ml |

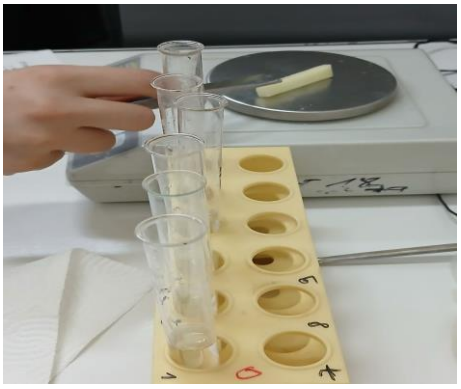
- **Preparing samples of potato:**

**1-** With the help of knife cut 6 potato **strips** approximately equal-sized (the size of 5 cm x 0.5 cm x 0.5 cm).



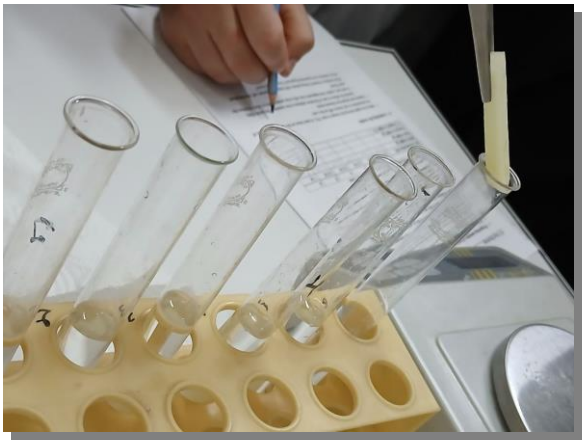
**2-** Dry them and record the initial weight (mass) of each potato strip using an electronic balance.

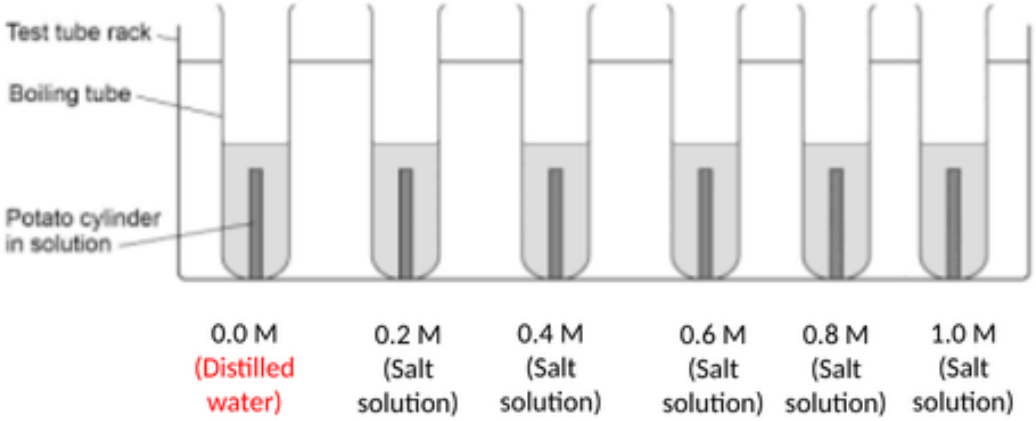




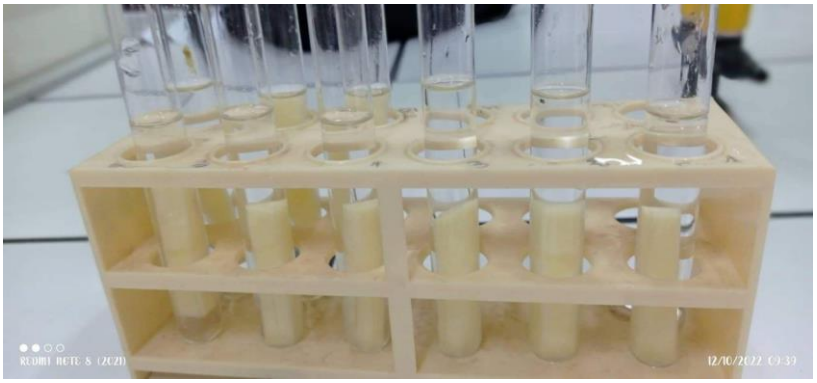
**3-** Note down the correct mass of each potato next to the correct concentration in the results table 2.

**4-** place them in test tubes containing each of the six solutions.





**5-** Leave the potato strips in their salt solutions for a set amount of time (example 30 minutes).



**6-** After 30 minutes, take each strips out of the Test tube, dry it on a filter paper (this removes any water before measuring its final mass).

**7-** Measure the new mass of each strip.

**8-** calculate the **percentage change in mass** of each strip and record the result in your table (Table 2). This can be calculated using the equation below:

**Percentage change in mass = ((final mass - initial mass) ÷ initial mass) x 100)**.

**Table 2:** Table showing change in the size and mass of the potato tissue

| **Tubes N°** | **1** | **2** | **3** | **4** | **5** | **6** |
|---|---|---|---|---|---|---|
| **Sal concentration (M)** | 0 M | 0.2 M | 0.4 M | 0.6 M | 0.8 M | 1 M |
| **Initial weight (g)** | | | | | | |
| **Final weight (g)** | | | | | | |
| **Change in weight (g)** | | | | | | |
| **% Change in weight (%)** | | | | | | |

## IV- Analysing the results

.

- Make a line graph showing the change in mass **(%)** of plant tissue on the y-axis as a function of the concentration of salt solution **(M)** on the x-axis
- Comment and interpret the obtained results:
  - Describe the effects of each of the below solutions on the masses of the potato disks.
  - In each case, explain what happened to the cells in the potatoes and in which direction the water flowed.
  - Which solution is closest to being isotonic with respect to a potato cell concentration?
  - Which solutions were hypertonic/hypotonic? How do you know?

# PW N°5: Transpiration Rates Using a Vesque Potometer.

**AIMS**:

- To demonstrate the process of transpiration (Water loss) in plant.
- Explain how a potometer works?
- Estimation of the rate of transpiration in small twigs using potometer.
- Exploring the link between different abiotic factors (temperature and wind) and transpiration rate.

The loss of water from the leaves of a plant causes water to be absorbed by the plant and moved through the xylem vessels to the leaves. The evaporation of water from the leaves (mainly through the stomata) to the atmosphere is called **transpiration**. During transpiration, water evaporates from the leaf. As a result, new water will be taken up by the plant to replace the evaporated water.

On the same principle, we can investigate the effect of different environmental conditions (such as temperature, humidity, light intensity and wind movement) on the **rate of transpiration** in small twigs is using a device called **potometer** (potos: drink, meter: measure) means a device that measures the water taken in by a plant.

The theory is made that the rate of evaporation from the leaf is equal to the rate of uptake. This will move the air bubble along the tube, towards the opening that contains the plant. The rate of transpiration is measured by tracking the distance travelled by the air bubble in graduated pipette per unit time.

**II. MATERIAL REQUIRED :**

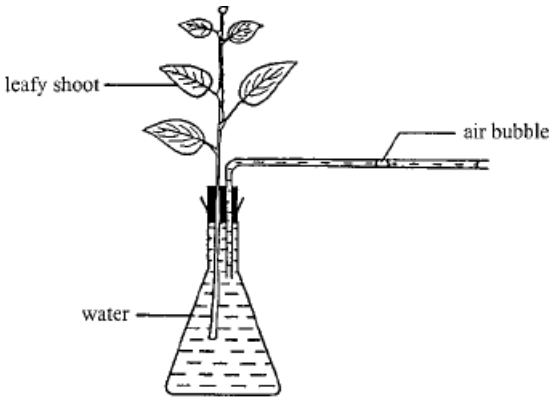
- Potometer
- Freshly cut plant shoot (leafy twig)
- Hair dryer
- Scissors/ Cecateurs
- Weight balance
- Parafilm paper
- Timer
- Distilled water
- Erlenmeyer Flask (250 ml)

**III. PROCEDURE:**

8. Cut a leafy twig from a plant.
9. Record the weight (Wp) of the twig.

**10.** Place the twig in the potometer and close the Erlenmeyer of the potometer with the cap (rubber stopper).

**11.** Set up the apparatus shown in the diagram below.




**12.** Smear all the connections of the potometer with parafilm paper to prevent water leakage.

**13.** Dry the leaves of twig with a hair dryer (as is, in a warm, windy place).

**14.** Mark or record the starting point of the air bubble (the position of water in the graduated pipette).

**15.** Record the initial time since start of experiment ($t_0$ = 0min)

**16.** Record the position of water (V1, V2, V3...) (Volume of water transpired) in the graduated pipette every 05 minutes for 30 minutes (T1, T2, T3...) and record the final position of the air bubble.

**17.** Note down in the results table (Table 1)

| Time (min) | **0** | **05** | **10** | **15** | **20** | **25** | **30** |
|---|---|---|---|---|---|---|---|
| position of water in graduated pipette (mm) | | | | | | | |
| Volume of water transpired (mm) | | | | | | | |

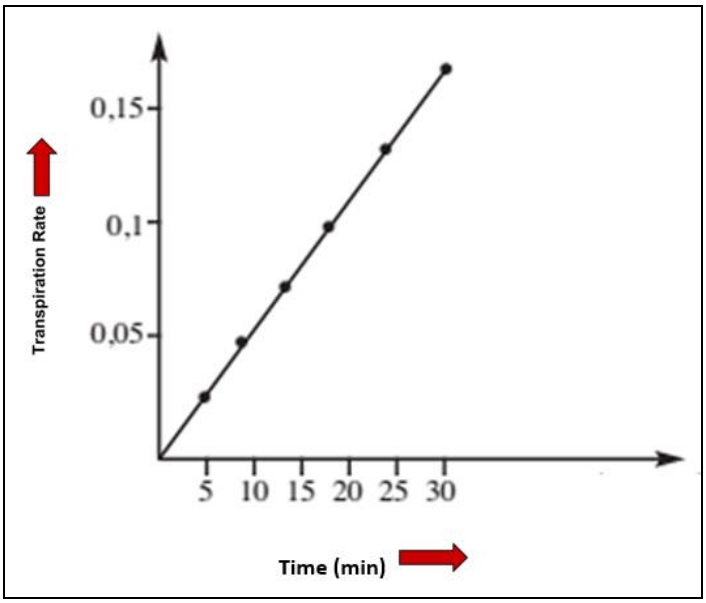
- The experiment can be repeated with different environmental conditions.

**IV- Results**

- Define the process of transpiration and Intensity of Transpiration.
- Calculate the Rate and Intensity of Transpiration
- Plot a line graph representing the change in water volume (water loss) (**mm**) as a function of time (**min**) (the effect of wind on the rate of transpiration)
- Comment and interpret the obtained results
- What conclusions can you make from the results above?

**Explanation for teachers**

1. Graphic representation:




Distance moved by bubble =Volume of water transpired (V)

In this activity the **rate of water uptake**, due to **transpiration**, by a shoot from a woody plant, is measured by timing how long a bubble takes to move a set distance, in the capillary tube of a **potometer**.

the rate of movement of the air bubble, i.e. distance travelled by the air bubble per unit time, can reflect the rate of water uptake by the leafy shoot. This is an indirect measurement of the rate of transpiration.

2. To calculate the rate of transpiration, divide the distance the bubble travelled by the set period of time. An example of the units your result can be is millimetres per minute (cm/min).

Rate of transpiration = Distance moved by air bubble (m)/ Time (min)

Rate of transpiration = water loss at time 2 - water loss at time 1/ Time 2-Time 1

$$Rt = \Delta V/\Delta T$$

**Example**

Estimate the rate of transpiration following a potometer experiment where it took the air bubble to move a distance of 40mm in 20 minutes.

- **Substitute the values into the formula.**
  40 / 20 = 2 mm per minute

Intensity of transpiration (I) = volume of water lost (mm) per unit of time (hour) by 1g of fresh weight

$$I = \Delta V/\Delta T/ Wp = mm/h/ 1g$$

With an accurate electronic balance, the loss of water from a leaf twig in a fixed period (e.g., 1 hour) can be recorded.

# PW N°6: Effect of wind on Transpiration Rates using Weight Potometer.

**AIMS**:

- To demonstrate the process of transpiration (Water loss) in plant.
- Explain how a potometer works?
- Estimation of the rate of transpiration in small twigs using potometer.
- Exploring the link between different abiotic factors (temperature and wind) and transpiration rate.

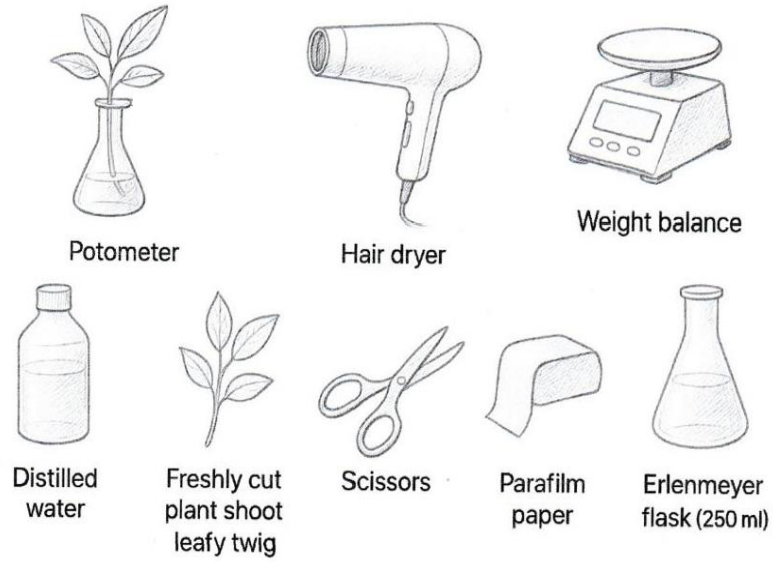
The loss of water from the leaves of a plant causes water to be absorbed by the plant and moved through the xylem vessels to the leaves. The evaporation of water from the leaves (mainly through the stomata) to the atmosphere is called **transpiration**. During transpiration, water evaporates from the leaf. As a result, new water will be taken up by the plant to replace the evaporated water.

On the same principle, we can investigate the effect of different environmental conditions (such as temperature, humidity, light intensity and wind movement) on the **rate of transpiration** in small twigs is using a device called **potometer** (potos: drink, meter: measure) means a device that measures the water taken in by a plant.

The theory is made that the rate of evaporation from the leaf is equal to the rate of uptake. Transpiration rates can also be measured using a weighing method, by weighing how much water a plant loses over a period of time.

## II. MATERIAL REQUIRED:

- Potometer
- Freshly cut plant shoot (leafy twig)
- Hair dryer
- Scissors/ Cecators
- Weight balance
- Parafilm paper
- Timer
- Distilled water
- Erlenmeyer Flask (250 ml)
- Vaseline
- Basin or sink filled with water



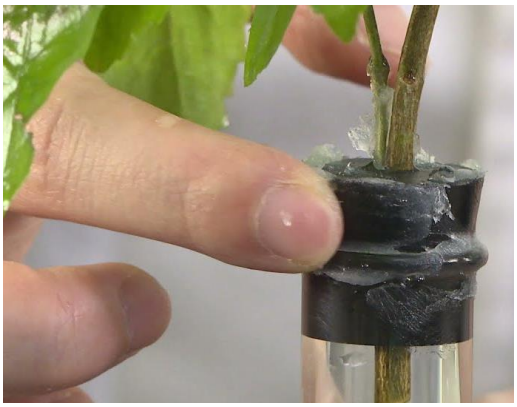
**III. PROCEDURE:**

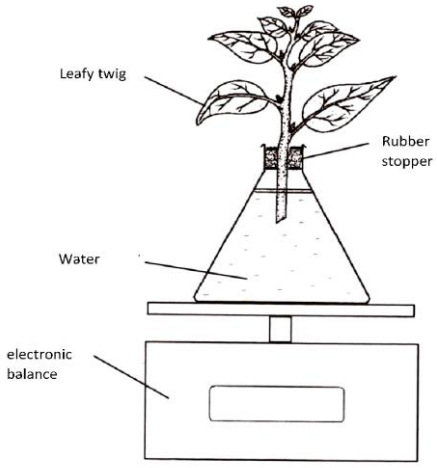
10. Take 2 similar cuttings from the plant using scissors. Try to get a length of stem about 8 cm long with several leaves at the top.
11. Cut a leafy twig underwater using a diagonal cut

* Diagonal cut creates a larger surface area for the uptake of water from a plant.

* Cutting underwater prevents air bubbles from entering the xylem where they could block the movement of water.

**1.** Set up the apparatus shown in the diagram below.






**2.** Smear all the connections of the potometer with parafilm paper or Vaseline to prevent water leakage.

**3.** Place the apparatus onto the electronic balance.

**4.** Adjust the environmental conditions: For example, dry the leaves of the first twig with a hair dryer to create windy conditions and leave the second twig in the laboratory conditions (still air).

**5.** Record the initial time since start of experiment ($t_0$ = 0min)

**6.** Mark or record the internal weight of the apparatus so that results can be expressed in the form of mm water lost per minute.

**7.** Record the weight of water (V1, V2, V3...) (Volume of water transpired) from the electronic balance every 05 minutes for 30 minutes (T1, T2, T3...).

* Water has a density of 1 gram per cubic centimeter. Therefore, 1 mm of water has a mass of 1 gram.

**8.** The table below shows typical results for using a weight potometer to compare water loss (transpiration rate) in two different wind levels (Table 1)

**Table 1:** water loss (transpiration rate) in two different wind levels.

| Time (min) | **0** | **05** | **10** | **15** | **20** | **25** | **30** |
|---|---|---|---|---|---|---|---|
| weight of the apparatus in still air (g) | | | | | | | |
| Volume of water transpired in still air (mm) | | | | | | | |
| weight of the apparatus in windy conditions (g) | | | | | | | |
| Volume of water transpired in windy conditions (mm) | | | | | | | |

The experiment can be repeated with different environmental conditions.

**IV- Results**

- Plot a line graph representing the change in water volume (water loss) (**mm**) as a function of time (**min**) in still air
- On the same graph, plot a line graph representing the change in water volume (water loss) (**mm**) as a function of time (**min**) in windy conditions (the effect of wind on the rate of transpiration).
- * Using a weight potometer to compare transpiration rates

| **Environmental condition** | **weight of the apparatus (g)** | | **Difference in mass (g)** | **Time (min)** | **Rate of Transpiration ml/min** | **Intensity of Transpiration ml/min/g** |
|---|---|---|---|---|---|---|
| | **At start** | **At end** | | | | |
| No wind (still air) | | | | | | |
| windy conditions | | | | | | |

- Comment and interpret the obtained results:
- Describe and explain the difference in water loss in the two conditions.
- Define the process of transpiration and Intensity of Transpiration.
- Estimate the rate of transpiration under different environmental conditions.
- Calculate the Intensity of Transpiration in the two conditions.
- Compare the transpiration rates in the different conditions.
- What conclusions can you make from the results above?

# PW N°7: Extraction of leaf pigments and separation by paper chromatography.

**AIMS**:

- To extract pigments from leaves and distinguish them by thin-layer chromatography (TLC).
- Isolate and identify photosynthetic pigments in plant leaves
- identification of the pigments through the calculation of their $R_f$ values and observations of their colour.

For photosynthesis to transform light energy from the sun into chemical energy in plants, the pigment molecules absorb light to power the chemical reactions. There are many types of photosynthetic pigments, but the two main groups are chlorophylls (Chlorophyll A and B) and carotenoids (which are further split into two classes: carotenes and xanthophylls).

In order to view and distinguish the primary four plant pigments, a simple technique known as chromatography can be used.

## II. MATERIAL REQUIRED:

- Spinach leaves - Mortar and pestle
- Scissors - Spatula
-Graduated cylinder. - Filter paper strips
- Erlenmeyer Flask - Funnel - Dropper
-Chromatography paper - Pencil - Hair dryer
- Acetone - Petroleum ether - Cyclohexane or Benzene

## III. PROCEDURE:

### I. Extraction of Pigments:

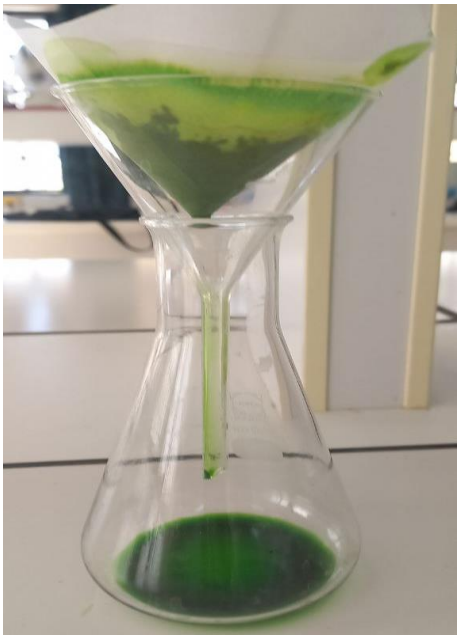
**1. Principle**

Leaves contain pigmentation which can be extracted and analyzed. The extraction process requires the crushing of a leaf and extraction of pigments with a suitable solvent.

**2. Preparing pigment extract :**

1. Pick a few fresh and green leaves of spinach and wash it.
2. Cut out small pieces of spinach using scissors.
3. With the help of mortar and pestle, grind the spinach leaves into a smooth paste


4. Gradually add 10 ml of acetone into the mortar and continue to grind with the pestle
5. Take the extract in a small separating funnel.
6. Rinse the grinding device with another 5ml solvent



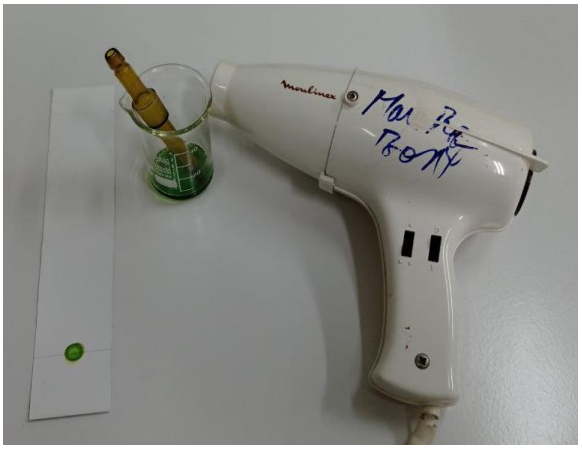
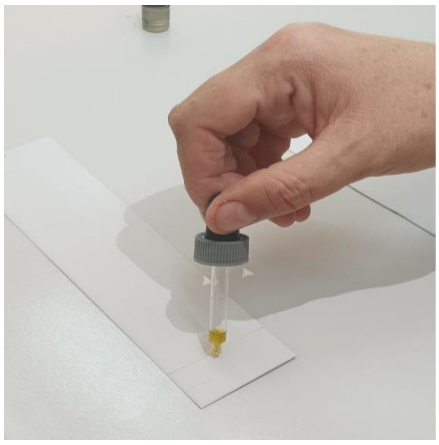
7. After filtration, a raw solution of pigments is obtained.

## II- Separation of Chlorophyll Pigments by thin-layer chromatography (TLC)

**Principle**: The process of chromatography separates molecules because of the different solubilities of the molecules in a selected solvent. In paper chromatography, the solvent carries the dissolved pigments as it moves up the paper. The pigments are carried at different rates because they are not equally soluble.

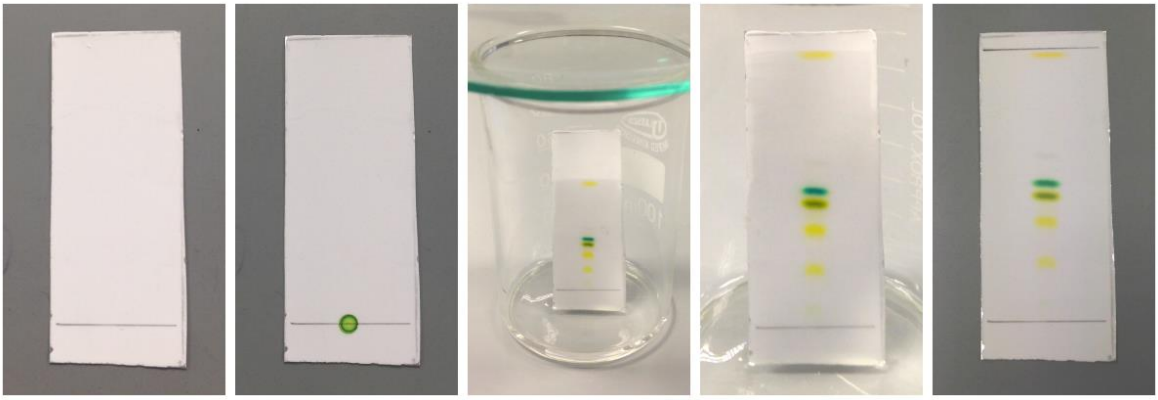
A pigment that is the most soluble will travel the greatest distance and a pigment that is less soluble will move a shorter distance.

1. Take a strip of chromatography paper approximately 18 cm long.
2. Then, carefully draw a pencil line 2 cm from the bottom of the chromatography paper.
3. Spot a little amount of leaf extract using a dropper repeatedly onto the center of the line
4. Dry each spot with a hair dryer.
5. Repeat the spotting and drying processes for 8-10 times to obtain a small area of concentrated pigment extract.

6. Prepare 10 ml of eluent (solvent) with: 85% petroleum ether, 10% acetone and 5% cyclohexane or benzene.
7. Pour the organic solvent into the graduated cylinder.

8. The solvent should cover the lower end of the paper but without touching the spot
9. Allow the chromatogram to develop until the solvent front has moved up to about 1 cm from the upper end of the paper (this will take approximately 15–30 minutes).



10. Take out the chromatogram. Trace the solvent front with a pencil before it evaporates and disappears.
11. Photograph the chromatogram as soon as it is dry. The colors will fade within a few hours. Print out a copy of the chromatogram for your report.
12. Use a pencil to circle each spot.

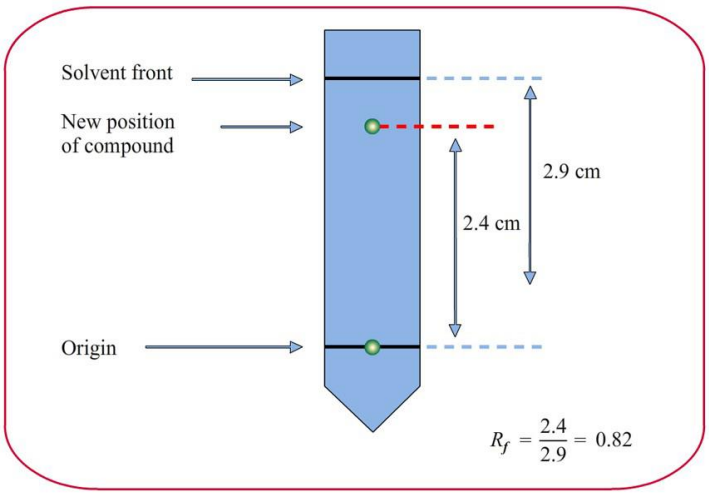
## IV- **Results**

13. Identify the pigments by their colors and their relative positions on the chromatogram.
14. Measure the distance from the first pencil line to the solvent front (the distance traveled by the solvent front).
15. Measure the distance from the pencil line to the middle point of each color band

*The retention factor ($R_f$) is the ratio of the distance traveled by a colored band to the distance traveled by the solvent front.

16. Calculate **$R_f$** values for each pigment using the following equation:

**$R_f$** = distance pigment travels (cm) / distance solvent travels (cm)



17. Using the chromatogram photo, try to work out how many pigments are present in the leaf extract (Use Table 1 in order to identify the pigments you have isolated).

Table 1: Different classes of pigments found in leaves and their respective colors.

| **Pigment** | **Colour** |
|---|---|
| Carotene | Yellow |
| Pheophytin a | Grey |
| Pheophytin b | Brown |
| Chlorophyll a | Blue green |
| Chlorophyll b | Green |
| Xanthophylls | Yellow |

18. Record your results in a table modeled after Table 2.

| Spot N° | Collor of the spot | Distance travelled by the pigment | Distance travelled by the solvent | $R_f$ Values | Name of the pigment |
|---|---|---|---|---|---|
| 1 | | | | | |
| 2 | | | | | |
| 3 | | | | | |
| 4 | | | | | |

- What conclusions can you make from the results above?

**SAFETY**

- Wear goggles and aprons when working with chemicals.
- Petroleum ether, acetone and alcohol are volatile and flammable. Avoid breathing vapors of the reagents.

# Explanation for teachers

**Observation**

- Sample leaves should be crushed into small pieces and put in a mortar for pestle grinding.
- Make sure the paper dips into the solvent but the spot of leaf extract doesn't by suspending it using a sticky tape.
- The dried paper strip displays four different bands. Discrete pigments can be distinguished with the help of colors.
- The solvent is allowed to run up the paper until it is close to the bung, at which point the paper is removed. The solvent's location is marked with a pencil before it evaporates and disappears., and the paper is allowed to dry.
- The final chromatography paper is known as a chromatogram, and it may be photographed to determine the exact position of each pigment.
- The color dissolves as the alcohol goes through the filter paper. Some pigments in the leaf travel more quickly than others because of their properties.
- The pigment's movement rate is measured by the Rf (retention factor) value. Rf value = distance transported by pigment from origin to center of pigment spot/distance from the origin to the solvent front. By applying this formula, you can determine the Rf value.
- The paper chromatography separates the pigments in the leaf based on the distance travelled by pigment molecules on the paper in a nonpolar solvent. Along with the alcohol, the pigments also migrate along the strips of paper.

**Chromatography Conclusion**

The pigments are light-absorbing molecules and are separated by using paper chromatography techniques in the lab. The pigments move on the paper based on their solubility in the solvent. Along with the alcohol, pigments also migrate along the strips of paper. Some pigments in the leaf travel more quickly than others because of their properties.

Carotene is identified as having the lowest molecular weight by its yellow to orange tint near the top of the paper. In the pigment separation of chlorophyll, chlorophyll a may be distinguished by its blue or dark green hue. When chlorophyll b pigments are separated, the color yellow-light green identifies chlorophyll B. In the chromatography solvent, xanthophyll is more soluble since it has gone up the paper. This describes the conclusion of paper chromatography.

# PW N°8: Estimation of plant Pigment Concentration by Spectrophotometer.

**PRINCIPLE**:

The procedure for estimation of chlorophyll content in plant tissue is based on the absorption of light by chlorophyll extracts prepared in aqueous acetone (80%). The simplest instrument for determining the concentration of chlorophyll in extracts of various plant specimens is a spectrophotometer by measuring how much light they absorb at various wavelengths.

**Spectrophotometry** is an experimental technique that is used to measure the concentration of solutes in a specific solution by calculating the amount of light absorbed by those solutes.

The main aims of this PW are to determine and compare pigment concentration in tow selected plants.

## II. MATERIAL REQUIRED:

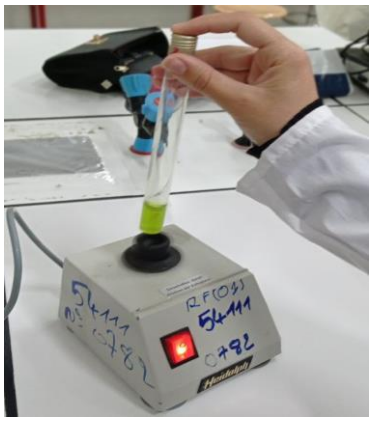
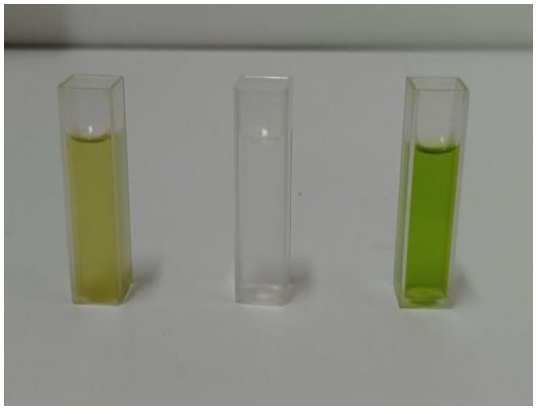
- Chlorophyll extracts of two different plants prepared by grinding 10g of leaf tissues in 20 ml of acetone (see PW N°3: Extraction of leaf pigments).
- Test tubes with caps.
- Spectrophotometer.
- Vortex Mixer
- Acetone 80%
- Pipettes
- Parafilm paper.
- Parafilm paper

## III. PROCEDURE:

Determination of Pigment Concentration: In order to determine the chlorophyll and carotenoid content of Chlorophyll extracts, it must measure the absorbance at several wavelengths, 470 nm, 645 nm and 663 nm.

1. Prepare extracts of two different plants by grinding 10g of leaf tissues in 20 ml of acetone (see PW N°3: Extraction of leaf pigments).

2. Put 1 ml of Chlorophyll extracts of plant N1 in a test tube and complete with 9ml of acetone.
3. Place the tubes on the mixing platform of the vortex Mixer. Make sure they are securely fastened to avoid spillage.
4. Load the proper volume of the sample into the cuvette.

5. Set the wavelength on the spectrophotometer to 470 nanometers (nm)
6. Put in the blank (acetone) cuvette to "Blank" the spectrophotometer. The absorbance (ABS) should read zero or a negative number.
7. Remove the blank and insert the clean cuvette containing 2/3 of diluted pigment extract and read the absorbance (A) values at specified wavelength.


8. Record the values of absorbance.

*The absorbance is also known as the optical density (OD).

*If you get an outlying result (Error), dilute the sample and measure the absorbance again.

*Remember to use the blank for each new wavelength setting (press the 0.00 button on the screen. Wait for it to finish auto-zeroing).

9. Repeat the test procedure with 645 and 663nm wavelengths of light.
10. Repeat the above-given procedure for every sample (plant N2).

11. Record the absorbance of each of your samples at the indicated wavelengths on the Table below.

| Plant Name | Wavelength | Absorbance |
|---|---|---|
| 1……. | **470** | |
| | **645** | |
| | **663** | |
| 2……. | **470** | |
| | **645** | |
| | **663** | |

**IV- Results**

**Determination of Pigment Concentration**

1- Calculate the concentrations of each pigment in the leaves extract (in micrograms per milliliter of plant extract solution (µg/ml)) using the following formula:

Chlorophyll a (chl a) =12.7×A663 – 2.69×A645

Chlorophyll b (chl b) =22.9×A645 – 4.68×A663

Total Chlorophyll (mg/mL) = Chlorophyll a + Chlorophyll b.

Carotenoids = (1000×A470 – 1.82 × Chl a – 85.02 × Chl b) /198

where:

A645 = absorbance at a wavelength of 645 nm.

A663 = absorbance at a wavelength of 663 nm.

A470= absorbance at a wavelength of 470 nm.

*These equations are designed for the use when 80% acetone is the solvent.*

2- Note down in the results table (Table2)

**Table 1**. Average Concentration (µg/ml) of chlorophylls and carotenoids in the chosen plants

| Plant Name | Chlorophyll a (µg/ml) | Chlorophyll b (µg/ml) | Chlorophylls a and b (µg/ml) | Carrotenoids (µg/ml) |
|---|---|---|---|---|
| 1……..…... | | | | |
| 2……...……. | | | | |

3- Comment and interpret the obtained results by comparing the differences in the pigment concentration in plant leaves.

4- What conclusions can you make from the results above?

# PW N°9: Evolution of carbon dioxide during respiration.

**AIM**:

- To demonstrate experimentally the liberation of carbon dioxide and consummation of O2 during aerobic respiration.

**THEORY**:

Respiration is a catabolic process wherein food is oxidized to release energy for various life processes. It is of two types, namely (i) aerobic respiration that takes place in the presence of oxygen, and (ii) anaerobic respiration that takes place in the absence of oxygen. In aerobic respiration the breakdown of food (glucose) leads to the release of carbon dioxide gas, water and energy in the form of adenosine triphosphate (ATP). In this experiment, we shall study the liberation of carbon dioxide and and consummation of O2 gas during an aerobic respiration.

## I. MATERIAL REQUIRED:

- Germinating seeds
- Erlenmeyer Flask (Conical flask)
- Rubber cork with one hole
- U-shaped delivery tube (tube bent twice at right angles)
- A small test tube
- Cotton wool or moist blotting paper
- Thread
- 20% Potassium hydroxide KOH solution
- Distilled water
- Vaseline
- Beaker.

## II. THEORY:

Respiration is a catabolic process which involves the breakdown of complex substances into simpler substances with a simultaneous release of energy. The process of respiration involves the utilization of oxygen with the release of carbon dioxide. Germinating seeds have a high rate of respiration which can be calculated by using Ganong's respirometer.

The general equation for respiration is:

$$C6H12O6+6O2 \rightarrow 6CO2+6H2O+ \text{energy}.$$

In the experiment, moist gram seeds are taken as they are actively respiring and releasing CO2. The CO2 released is absorbed by KOH and forms K2CO3.

$$2KOH + CO2 \longrightarrow K2CO3 + H2O$$

**III. PROCEDURE:**

1. Take about 25-30 seeds of gram and germinate these seeds by placing them on moist cotton wool or moist blotting paper for 3-4 days.
2. Place the germinated seeds into a conical flask on a cotton wool or moist blotting paper.
3. Take freshly prepared 20% KOH solution in a small test tube and hang it in conical flask with help of thread.
4. Close the mouth of conical flask by placing a rubber cork containing one hole.
5. Through the hole of rubber cork, insert one end of the U-shaped glass delivery tube in the conical flask and place the other end into a beaker filled with water as shown in Fig.1.
6. Seal all the connections of the experimental set-up with Vaseline so as to make it air-tight so that the gas evolved during the process of respiration by the germinating seeds does not leak out.
7. There will be a rise of water level inside the delivery tube at the end dipped in the water due to capillary action.
8. Mark the initial level (h1) of water in the U-shaped delivery tube with a sketch pen.
9. Keep this set-up undisturbed for about forty-five minutes in the bright sunlight.
10. Note and record the final water level (h2) in the delivery tube.
11. The amount of O2 consumed by respiring material /hr can be calculated by the formula:

Volume of O2 consumed= Final column reading (ml) – Initial column reading (ml).

Rate of Respiration = Volume of O2 consumed (ml)/ Time (hour) x Amount of respiring materials (mgs)

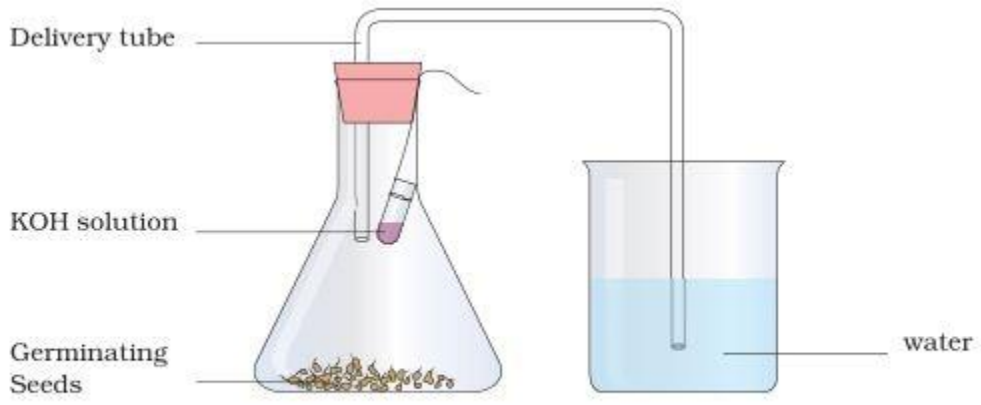



**Fig:** Production of carbon dioxide gas during respiration in conical flask

**Inference:**

Due to respiration of seeds, CO2 has been released along with consumption of O2. The CO2 is then absorbed by KOH and a partial vacuum is produced due to O2 utilization. As such, water upward into the tube. The rise corresponds to the volume of O2 consumed.

**IV- OBSERVATIONS:** After sometime, the level of water in U-shaped delivery tube dipped in water of the beaker rises.

**V-RESULTS**

• Analyse the experimental results and give reasons for the results obtained.

• What is the role of KOH in this experiment?

• Why do we use germinating seeds in this experiment?

*** NOTE FOR THE TEACHER**

- In germinating seeds carbohydrate is usually the main respiratory substrate and respiratory quotient is usually 1 (as the amount of C02 released and 0 2 absorbed is equal).
- Germinated seeds in a conical flask release CO2 during respiration. The CO2 released is absorbed by KOH present in the hanging test tube in conical flask.
- the germinating seeds release CO2 during respiration and requires large amount of O2 which creates a pressure inside the conical flask due to which the water is sucked in upward direction.
- This creates a vacuum in conical flask So an equal volume of water rises up in the delivery tube leading to change in level of water in the delivery tube.
- This indicates that the germinating seeds are actively respiring and evolving carbon dioxide gas during the process of respiration.
- This experiment can also be performed using flower buds.
- The rate at which the KOH solution rises can be calculated by measuring the volume of oxygen consumed per unit time per ml of the respiring material during aerobic respiration.

**VI- PRECAUTIONS:**

- Germinating seeds should be kept moist.
- All connections of the set-up should be air-tight.
- Freshly prepared KOH solution should be used.
- KOH is corrosive. Handle it carefully.
- Keep one end of U-shaped delivery tube in conical flask and the other end immersed in water of the beaker.
- The test tube containing KOH should be hung carefully.

# PW N°10: Seed Germination Rate.

## INTRODUCTION

Seed germination is the fundamental phenomenon that determines the successful growth and development of each plant species. It is regulated by several environmental factors, such as temperature, soil moisture, soil pH, light intensity, and photoperiod.

Germination is a critical stage in the life cycle of plants. It starts with the uptake of water by the dry seed (imbibition), continues with biochemical and physiological preparative processes and the elongation of the embryonic axis, and usually terminates with the protrusion of the radicle out of the seed. Three different stages can be distinguished during seed germination: (i) Imbibition, during which water absorption by the seed takes place (ii) Initiation of biochemical and meristematic activities take place (iii) Seed coat rupturing permits the emergence of radicle and shoot. The seed's physiology is then activated, and it begins to respire.

**AIM**:

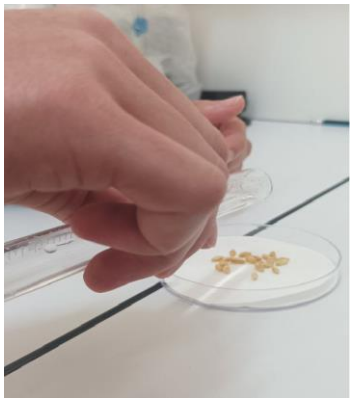
The germination test of a seed lot aims to determine the germination capacity (%), the rate of germination of wheat seeds or others (lentil, bean ...) under different conditions of humidity and light in the laboratory ambient temperature.

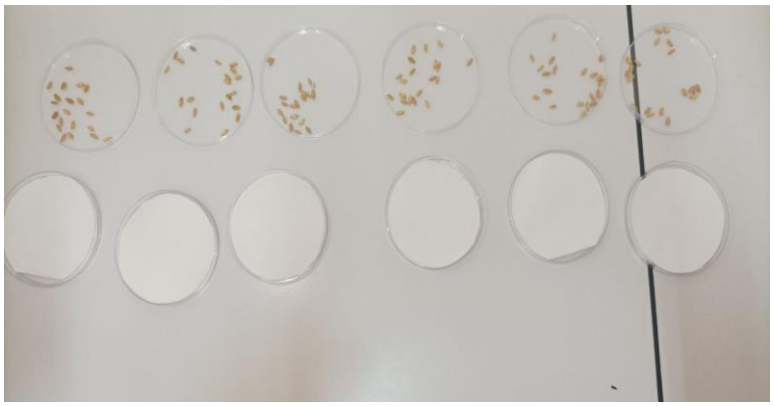
## II. MATERIAL REQUIRED:

- Wheat seeds or others (lentil, bean ...)
- Graduated pipette
- Petri dishes
- filter paper (Whatman n°1)
- Distilled water

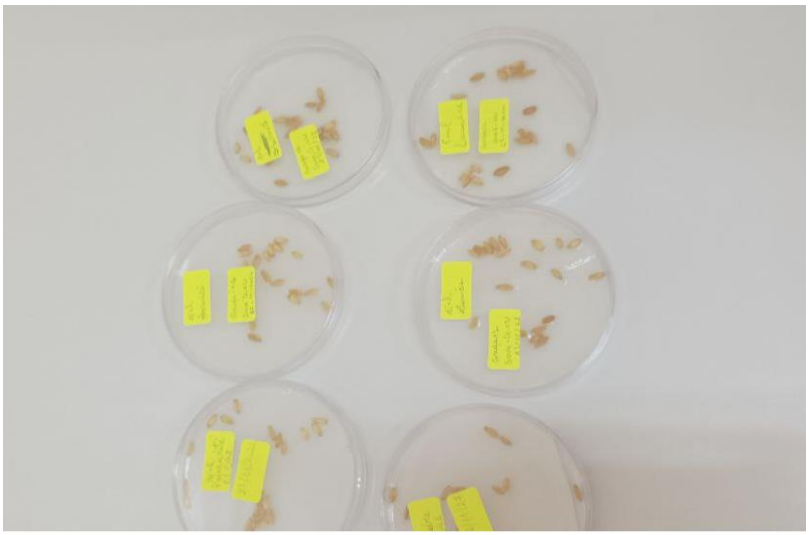
## III. PROTOCOLE EXPERIMENTAL

- Take 6 Petri dishes
- Put two layers of filter paper (Whatman n°1) with a diameter equal to that of Petri dishes
- Put 20 wheat seeds in each petri dish.





- With a pipette, add the water at different volumes (5ml, 15ml, 30ml) in two repetitions per volume.
- Put the boxes of the different volumes of water in daylight (Exposed to light) and in the cupboard (at darkness).
- Using the marker, write the n° of the experiment (see result table) and a name or symbol allowing to recognize the boxes (Petri dishes).

- Note the start date of the test as well as the number of seeds used for the test.
- Indicate each day how many seeds have germinated during 7 days.
- A seed was considered as germinated when the length of the radicle exceeded 1mm.
- Calculate the mean percentage germination of the accession from the results of all the replicates.


- Record the Number of germinated seeds and the Capacity of germination in your table (Table 1):

**Table 1**: Number of germinated seeds and the Capacity of germination (Cg%) under different water levels (5ml, 15ml, 30ml) and at different light conditions (daylight and at darkness)

| Conditions | Humidity (ml) | Light Conditions | Day 1 | | Day 2 | | Day 3 | | Day 4 | | Day 5 | | Day 6 | | Day 7 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | NGS | Cg (%) | NGS | Cg (%) | NGS | Cg (%) | NGS | Cg (%) | NGS | Cg (%) | NGS | Cg (%) | NGS | Cg (%) |
| 1 | 5 | Cupboard (Darkness) | | | | | | | | | | | | | | |
| | | Exposed to light | | | | | | | | | | | | | | |
| 2 | 15 | Cupboard (Darkness) | | | | | | | | | | | | | | |
| | | Exposed to light | | | | | | | | | | | | | | |
| 3 | 30 | Cupboard (darkness) | | | | | | | | | | | | | | |
| | | Exposed to light | | | | | | | | | | | | | | |

***NGS** is Number of germinated seeds ***Cg (%)** Capacity of germination (%)

- Germination capacity (Cg) **(%)**: This is the percentage of seeds capable of germinating under well-defined conditions. Seed germination percent was determined by observing the number of seeds that germinate at the given days of observation. The capacity of germination is determined, according to the formula:

Cg **(%)** = (Number of germinated seeds / Total Number of seeds) x 100 **= (NGS/TNS) x 100**

Wher:

**Cg (%)** is Capacity of germination, **NGS** is the Number of germinated seeds, **TNS** is Total Number of seeds.

The kinetics of seed germination under the effect of several environmental factors can be illustrated by the **curves** of germination. The analysis of this kinetics shows a **first** phase of latency, due to the imbibition of the seeds, a **second** linear phase in which there is an acceleration of germination, and finally a **third** phase characterized by plateau indicating a phase of germination stability.

**Résultats**

- Represent the results graphically as curves of germination representing the change in germination capacity (Cg) (**%**) as a function of time (**Days**) at different experimental conditions:

  1. Impact of different water levels (5ml, 15ml, 30ml) on germination capacity (%) at light conditions (daylight and at darkness) at the given days of observation.

  2. Effect of light on seed germination capacity (%) at different water levels (5ml, 15ml, 30ml).

- Comment and interpret the obtained results:
- Describe and explain the Impact of different water on germination capacity (%).
- Describe and explain the Impact of light on germination capacity (%).
- What conclusions can you make from the results above?

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