Laboratory Manual

BTS309: Physiology and Ecophysiology of Plants

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Contribution from VI Semester 2019 Batch

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Contents

Given a set of numbers, there are elementary methods to compute its Greatest Common Divisor, which is abbreviated GCD. This process is similar to that used for the Least Common Multiple (LCM). This second section may include some special word, and expand the ones already used.

Friday, 16 February 2019

1 Plant-water relation

1.1 Aim

To determine water potential of a plant tissue by plasmolytic method.

1.2 Theory

Concept of water potential is important because plant water condition determines a large extent their general condition, their health and growth. Solute potential alone is not enough to predict the movement of water through plant potential. If water moves into the cells. The cell burst by endosmosis and if water moves out which is exosmosis, the plant will shrink. Water potential concept is used to predict the movement of water in and out of the cell (Xanthopoulos et al., 2006).
Gravimetric method is used to determine water potential by weighing the tissue samples before and after incubation in different concentration of solute to determine the movement of water through difference in concentration. The water potential of the tissue is considered equal to osmotic potential of the incubating solution at which there is no change in tissue weight (Saupe, 2009).

Plants use water potential to transport water to the leaves so that photosynthesis can take place. Water always moves from the system with a higher water potential to the system with lower water potential. The difference between the free energy of water molecules in pure water and energy of water in any other system is termed water potential (Xanthopoulos et al., 2006).
If a sample of tissues immersed in a solution of varying water potential (concentration of water molecules), the cells will lose or gain water by osmosis. If the cell is put into solution of higher water potential (hypotonic solution), water will go inside the cell. As the pressure potential rises inside the cell it becomes turgid. In the plant cells turgidity starts rising up the moment when cell can't take more water because of the cell wall. If the cell is put into solution of lower water potential (hypertonic solution) the water will go out of cell and cell will be plasmolysed. In both ways cell will gain or lose mass (Xanthopoulos et al., 2006)

1.3 Hypothesis

When cells are put in a solution with a different water potential than inside of them, cells will gain or lose water. If concentration of solution is the same, there will be no change in mass and the water potential of the plant tissue is usually negative and can never be greater than zero (Aske & Iqbal, 2014).

1.4 Materials and Method

1. Prepared 1 M stock solution by dissolving 85.5 g of sucrose in 250 ml of distilled water.
2. 1M sucrose and distilled water was used and we prepared a series of 10cm3 sucrose solution in test tube of different concentrations : 0.1M, 0.12M, 0.14M, 0.16M, 0.18M
3. Test tubes were labelled according to their concentrations.
4. Sphere shape of leaves Rumex nepalensis was made using cork borer of same diameter
5. Weighed each group of leaf tissue before placing in the test tube and recorded their readings
6. Three discs of leaves were placed in each test tube.
7. Covered the test tubes using sealing film.
8. The set up was left for one hour undisturbed.
9. The tissue was removed and measured their final weight.
10. The percentage change in weight loss was calculated using the formula;
11. Change in weight=final weight- initial weight/initial weight*100.
12. Using the data from the table the graph was plotted.

1.5 Observation table

Sl.no	Molar concentration	initial weight	final weight	change in weight	% change in weight
1	0M	0.321	0.354	0.033	10.3
2	0.15M	0.289	0.31	0.021	7.3
3	0.2M	0.281	0.292	0.011	3.9
4	0.25M	0.278	0.291	0.013	4.7
5	0.3M	0.303	0.303	0	0.0
6	0.35M	0.311	0.3	-0.011	-3.5
7	0.4M	0.241	0.23	-0.011	-4.6
8	0.45M	0.285	0.276	-0.009	-3.2
9	0.5M	0.286	0.271	-0.015	-5.2
10	0.55M	0.283	0.27	-0.013	-4.6
11	0.6M	0.315	0.299	-0.016	-5.1

%

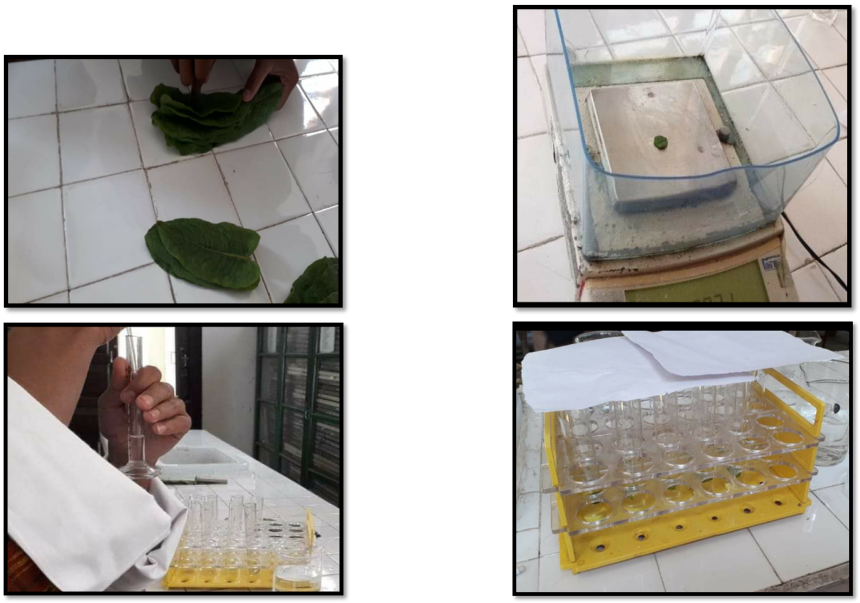


Figure 1: *Experimental set-up*

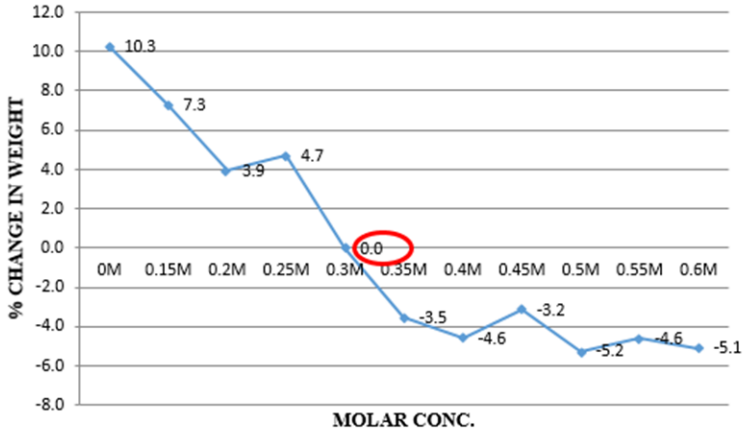


Figure 2: *Graph showing %change in weight against sucrose concentrations (M)*

1.6 Results and Discussion

The graph shows that as the molar concentration of sucrose increases, the % change in weight decreases. At pure water the change in weight was the highest. At the concentration 0.3 M, the change in weight was zero which means that there was no gain or loss of water from the potato tissues. Therefore, the solute water potential is:

Water potential = - iCRT, Given,

i = ionization constant which is 1 because sucrose does not ionize in water

C = Molar sucrose concentration at equilibrium which is 0.4M

R = Pressure constant (0.0831L bar/ mole K) and

T = Temperature in Kevin (273 + RT°C) which is 273+27°C = 300°C

Water potential = - (1*0.3*0.083*300)

= - 7.48 bar.

Water potential of the *Rumex nepalensis* tissue was -7.479 MPa at 0.2 M sucrose solution.

The weight of the leaf increased after keeping the leaf for an hour in the solution. It is because of osmosis which takes place from higher to lower concentration. The weight of the leaf increased with increase in concentration of sucrose solution.

At the concentration of 0.2 M of sucrose, the change in the weight is zero which means that there is no gain or loss of water from the leaf and this is the water potential of the leaf since there is no gain nor loss of water in the cell.

1.7 Precautions

1. The measurement must be accurate while measuring the tissues.
2. Leaf tissues with equal weight and diameter must be taken.
3. The weighing machine should always be tare to zero when weighing next object.
4. The experiment should be conducted as quickly as possible to avoid loss of water through evaporation.

Friday, 22 February 2019

1 Water potential of given tissue by plasmolytic method.

1.1 Aim

To determine osmotic potential and water potential of given tissue by plasmolytic method.

1.2 Theory

Osmosis is process of diffusion of solvent through a semipermeable membrane in the direction of the solution when it parted from pure solvent by a semipermeable membrane. The plasma membrane works as a differentially permeable membrane providing an ideal example of an osmotic system.The vascular and plasma membranes in plant cells work as a differentially permeable membrane and allow the water to pass freely but permit the passage of solutes is selective. Hence, the force with which pure water tend to move into the solution present inside the vacuole is known as osmotic pressure.

Living plant cell with higher concentration tends to draw water when limited by the cell wall, developing a turgor pressure, the pressure of the water on the wall. The real osmotic pressure develops as hydrostatic pressure as in the 'osmometer'. Osmotic value and actual osmotic pressure are different terms, where osmotic value is equivalent to chemical potential and not the osmotic pressure. As the equation;
(DPD = (OP - TP) can be replaced more practically by the equations
(S = W – P) where, S = the suction force,
W = osmotic value and
P = turgor pressure.
Statistically osmotic potential is equal to the osmotic pressure although given a negative sign. The osmotic potential of pure water is zero and it gets lowered (-ve) when the solutes are added. When two solutions are separated from each other by a semi-permeable membrane, the direction of the osmotic movement of water and osmotic pressure depends upon the temperature, pressure and the solute molecules on the two sides (Rastogi, 2011).

1.3 Hypothesis

The cell undergoes plasmolysis when the concentration of the solution outside the cell is greater than the concentration inside the cell.

1.4 Materials and Method

1. Prepared 1 M stock solution by dissolving 8.5 g of sucrose in 50 ml of distilled water.
2. Prepared stock solution by diluting to different concentration ranging from 0.10M, 0.12M, 0.14M, 0.16M, 0.18M, 0.20M, 0.22M, 0.24M, 0.26M, 0.28M and made the final volume 10 ml.
3. Peeled off the epidermal cell from the leaf and observed under a microscope.
4. Counted and recorded the number of epidermal cell observed.
5. Immersed all the epidermal cells in the varying concentration of sucrose solution.
6. The solution with placed epidermal cells was kept for 30 minutes.
7. After 30 minutes, observed the epidermal cell under microscope and counted the number of plasmolyzed cells and recorded.

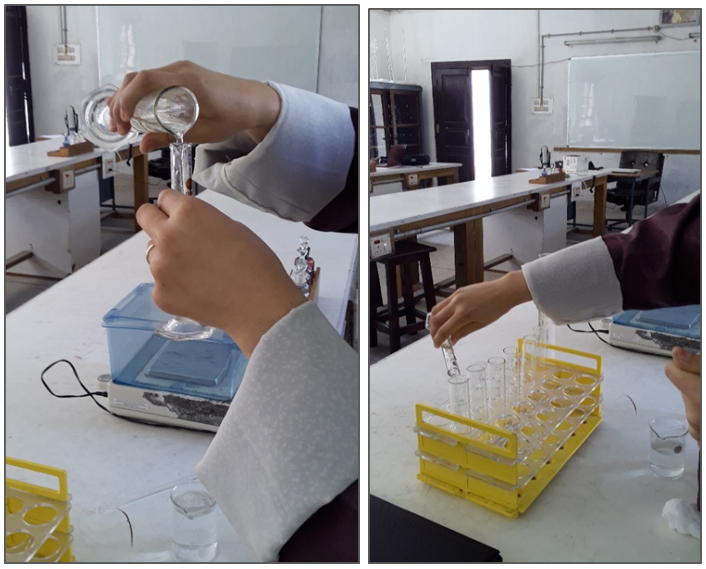


Figure 3: *Preparation of sucrose molar concentration*

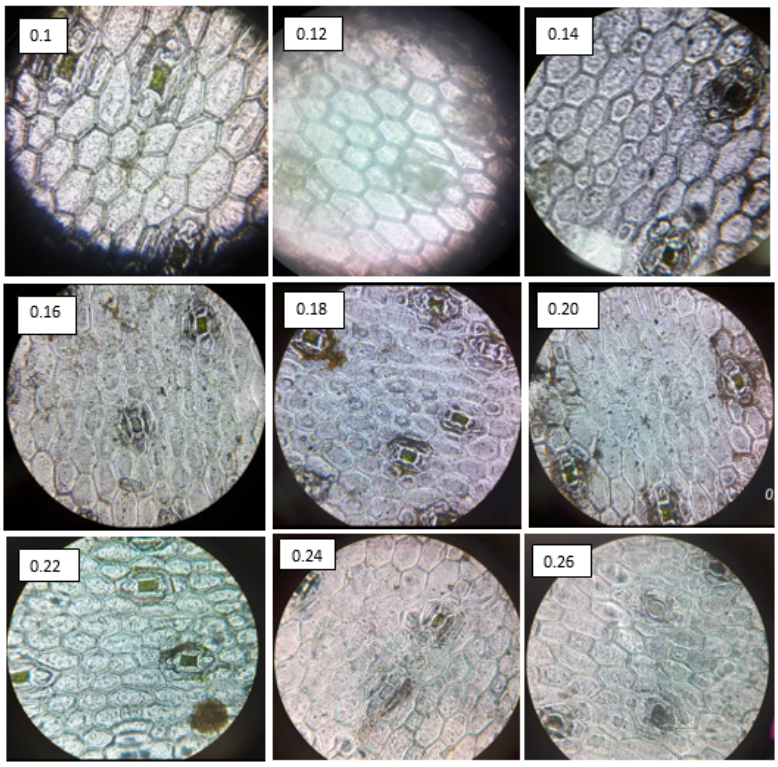


Figure 4: *Micrographs of cells in different concentrations of sucrose after 30 minutes of incubation*

1.5 Results and Discussion

1.6 Observation table

Concentration of sucrose solution	Cells plasmolysed	No. of cells not plasmolysed	% of plasmolysed cells
0.1	3	81	3.57
0.12	8	79	9.20
0.14	12	76	14.29
0.16	14	75	15.73
0.18	18	72	21.43
0.2	20	70	22.22
0.22	27	67	32.14
0.24	32	60	34.78
0.26	48	48	50
0.28	51	49	51

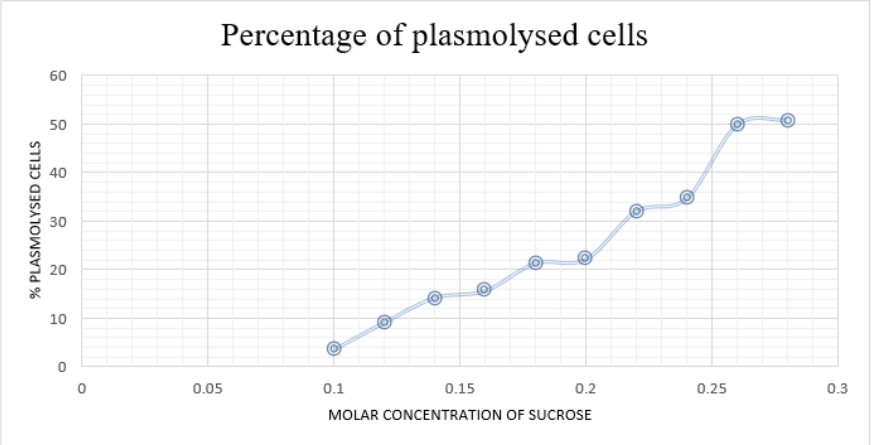


Figure 5: *Graph showing percentage plasmolyzed cells*

Calculations

Osmotic potential ($\Psi\pi$) using **Van't Hoff Law or equation**:
$\Psi\pi$ = -miRT
Where; m = Molality of sucrose (0.26)
i = ionization constant (1.0 for sucrose)
R = Raul's gas constant (0.083 liter bars/mole)
T = temperature in Kelvin (273+27) = 300
$\Psi\pi$ = - 0.26 * 1.0 * 0.083 * 300
= -6.474 bar

The number of plasmolyzed cells increased with the higher concentration of the sucrose solution, i.e., 0.1 concentration of sucrose had lowest number of plasmolyzed cells while 0.28 had the highest number of plasmolyzed cells.

In higher concentration of sucrose solution, more the number of plasmolyzed cells were observed. This was because of the exosmosis of the cells that took place in the hypertonic solution. The 0.1 concentration of sucrose solution had only 3 cells plasmolyzed out of 84 cells (3.75 %), while 0.28 concentration of solution had plasmolyzed cells out of 51 cells (50 %). The osmotic potential of the plant tissues was – 6.474 bar.

1.7 Precautions

1. Avoid counting errors of the cells under the microscope.
2. The time period of immersing cells in different concentration of the sucrose solution should be equal for all cells.
3. Accurate weighing of sucrose while preparing different concentration of it solutions.

Friday, 1 March 2019

1 Water potential concept

1.1 Aim

To determine water potential of a plant tissue by gravimetric method.

1.2 Theory

The energy status of the water in the tissue which is the main cause for the movement of water into and through plants along free energy gradients. This process affects the level of metabolic activity in a plant. The free energy status of water in plant cells can be expressed in terms of diffusion pressure deficit or DPT in the past (O'Leary, 1970).
The free energy status of the plant is determined by water potential. Therefore water potential is a measure of the energy state of water which are affected by dissolved solutes, pressure and matrix particles. The water potential by dissolved solutes is always negative whereby solutes decreases water potential. The pressure may be positive, negative or zero but it is mostly positive since most of plant cells remain turgid. According to Boyer, 1968, the water potential of the tissue affects the growth rate because of the role of turgor in cell enlargement. The direction of the water movement can be detected in two ways: a) by measuring the change in tissue such as length, weight or volume and b) measuring change in the solution concentration. This experiment basically deals with the change in the weight of the potato tissue in varying sucrose concentration (O'Leary, 1970).
Water potential values determined by this method may be slightly more negative. This occurs when the apoplast becomes infiltrated with water and solutes which result in increasing the weight of tissues. The technique of gravimetric method for measuring water potential is advantage of being simple to perform and doesn't require expensive equipment (Aske & Iqbal, 2014).

1.3 Hypothesis

The increase in the concentration of sucrose decreases the water potential of the potato tissue that leads to decrease in their weights.

1.4 Materials and Method

1. Stock solution was prepared by dissolving 34.45g of sucrose solution in distilled water making the final volume to 100 ml. The stock solution was again diluted in different concentration ranging from 0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8 and 0.9 in 10 labeled test tubes.
2. Took potato tuber and remove and remove the skin.
3. Then bored a hollow cylinder into the tuber and with the piston take out a cylinder of tissue.
4. Cut the cylindered potato into equal length of 10cm by measuring using ruler.
5. Placed the slice of tubers on the filter paper to blot off the water.
6. Initial weight of each slice of tuber was measure with the help of weighing machine and was recorded.
7. Placed each slice of tuber in labeled test tube with different concentration.
8. Kept the experimental set up for a hour.
9. The slice of potato tubers were then removed from the solution and excess sucrose was removed or blotted using filter paper.
10. The final weight of the slice of tubers was then noted. Change in the weight was calculated as follows:

1.5 Observation table

Sucrose molarity	Initial weight	Final weight	Change in weight	% Change in weight
0.00M	0.667	0.706	0.039	3.9
0.1M	0.643	0.674	0.031	3.1
0.2M	0.642	0.662	0.02	2
0.3M	0.64	0.666	0.026	-2.6
0.4M	0.64	0.636	-0.004	-0.4
0.5M	0.635	0.625	-0.01	-1
0.6M	0.635	0.623	-0.012	-1.2
0.7M	0.624	0.617	-0.007	-0.7
0.8M	0.606	0.593	-0.013	-1.3
0.9M	0.606	0.592	-0.014	-1.4

%

Water potential = - iCRT,

Given,

i = ionization constant which is 1 because sucrose does not ionize in water

C = Molar sucrose concentration at equilibrium which is 0.4M

R = Pressure constant (0.0831L bar/ mole K) and

Figure 6: *Experimental set-up*

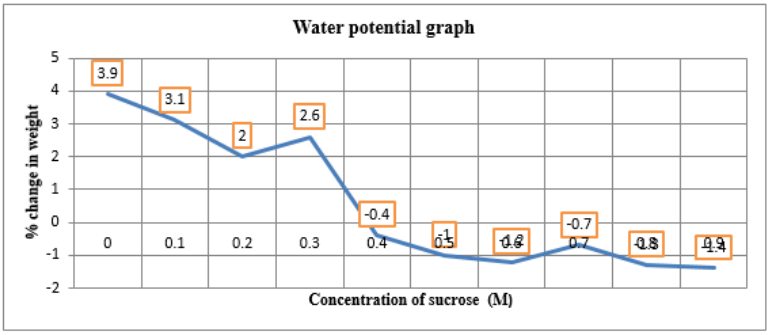


Figure 7: *Graph showing %change in weight against sucrose concentrations (M)*

T = Temperature in Kevin (273 + °C) which is 273+27 °C = 300 °C
Water potential = - (1*0.4*0.083*300)
= - 9.96 bar.

1.6 Results and Discussion

After keeping the set up for one hour, changes in the slice of tubers were observed in terms of weight of a slice of tubers. The slice of tuber which was placed in test tube containing only distilled water has increased its weight but the slice of tubers which were placed in sucrose solutions has shown decrease in their weight as increase in the concentration of sucrose solutions.
The molar concentration of the slice of tubers is 0.04M which shows the molar concentration of sucrose is equal to the slice of tuber water potential at 0.04M. Therefore, the water potential for the potato tissue obtained is -9.96 bars.
The increase in weight of potato tuber in distilled water is mainly due to absence of solutes that leads to endo-osmosis. The weight of slice of tuber decreased in sucrose solution due to presence of solutes that leads to exo-osmosis. The occurrence of negative results is due to increase in concentration of sucrose which leads to decrease in water potential.
Due to increase in concentration of sucrose solution, the diffusion pressure deficient increased that leads to lower in turgor pressure resulting in low weight in slice of potato tuber. The weights of tubers decrease due to increase in concentration of sucrose in Gravimetric method.

1.7 Precautions

1. The measurement must be accurate while measuring the tissues.
2. Leaf tissues with equal weight and diameter must be taken.
3. The weighing machine should always be tare to zero when weighing next object.
4. The experiment should be conducted as quickly as possible to avoid loss of water through evaporation.

1. The slice of tuber must be cut in equal length for uniformity.
2. In order to avoid the occurrence of error in the set up, the test tubes should be marked properly.
3. The slices of tuber should measure accurately before and after the set up to find the changes in their weights.
4. The excessive water and sucrose solutions must be blotted away by using blotting paper to avoid the occurrence of error in the measurements.

Friday, 8 March 2019

1 Demonstration of the law of limiting factors

1.1 Aim

To study the effects of light and carbon dioxide (CO_2) on oxygen (O_2) evolution during photosynthesis and demonstrate the law of limiting factors.

1.2 Theory

Photosynthesis is the use of light energy to decompose water and to transfer hydrogen from it to carbon dioxide in the presence of photosynthetic pigment, i.e. chlorophyll. Through oxidation- reduction reactions, sugars are produced.
It was F. F. Blackman in 1905 who first recognized that in photosynthesis where the "process is conditioned as to its rapidity by a number of separate factors, the rate of the process is limited by the pace of the 'slowest' factor" (Smith, 1938).

Factors affecting the rate of photosynthesis:

1. **Light intensity:** At low light intensities, as light intensity increases, the rate of the light-dependent reaction, and therefore photosynthesis generally, increases proportionately (straight line relationship). There is a linear relationship between incident light and CO_2 fixation rates at low light intensities. Light intensity is one of the most important environmental factors determining growth-rate, acting on the rate of photosynthesis both directly and indirectly. The direct and immediate effects of light intensity on photosynthesis have long been appreciated, while the importance of the indirect effects of the light intensity in which a plant is grown on its photosynthetic capacity has recently emphasized (Woledge, 1971).

2. **Carbon dioxide:** CO_2 is a trace gas in the atmosphere. From the time it came to be recognized that CO_2 was absorbed by the leaves during, photosynthesis and that with an increase in its concentration the rate of assimilation increased, attempts have been made to determine an optimum concentration of carbon dioxide, a percentage which may induce rapid photosynthesis, leading to increased growth in plants. An increase in the carbon dioxide concentration increases the rate at which carbon is incorporated into carbohydrate in the light-independent reaction, and so the rate of photosynthesis generally increases until limited by another factor (van den Honert, 1930).

3. **Temperature:** Although the light dependent reactions of photosynthesis are not affected by changes in temperature, the light independent reactions of photosynthesis are dependent on temperature. They are reactions catalysed by enzymes. As the enzymes approach their optimum temperatures the overall rate increases. It approximately doubles for every 10°C increase in temperature. Above the optimum temperature the rate begins to decrease, as enzymes are denatured, until it stops (Woledge, 1971).

Blackman's Principle of Limiting Factors This principle states that when a process is governed by more than one factor, the rate of the process is governed by that factor which is closest to its minimum value (Smith, 1938).

1.3 Hypothesis

Rate of photosynthesis is affected by more than one factor and rate is determined by factor which is at slowest pace.

1.4 Materials and Method

1. 2 types of water, tap water and pond water were collected.
2. Nasturtium officinale (water cress) was collected near fish pond.
3. Took 2 Wilmott's bubbler and filled with tap water and pond water in each bubbler respectively.
4. The roots of plants were cut off and inserted in Wilmott's bubbler tube.
5. Set up was made air tight in both bubblers.
6. Set up with pond water was kept outside in sunlight.
7. Set up with tap water was kept in shade.
8. Evolution of oxygen bubble at interval of 5 minutes was recorded and observed carefully by 2 people.
9. For tape water set up $NaHCO_3$ of 0.5 g, 1 g, 1.5 g, 2 g, 2.5 g was added after every 5 minutes.
10. Few minutes after the experimental set up, we observed oxygen bubbles evolution in both set up as shown below.

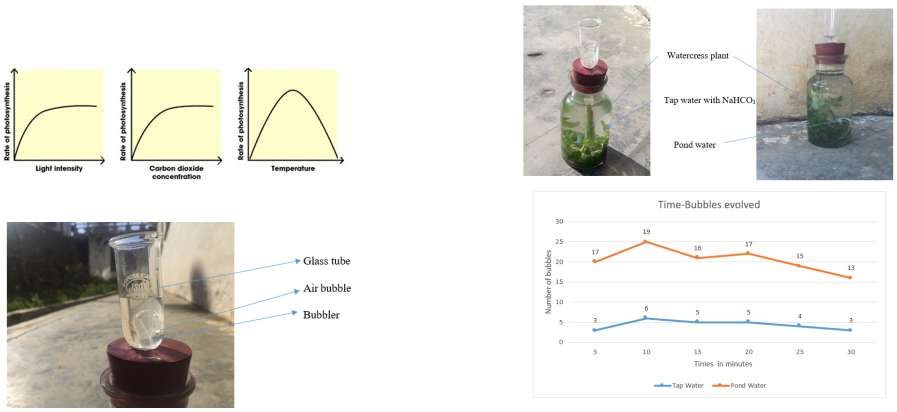


Figure 8: *Measuring rate of photosynthesis using Wilmott's bubble tube*

1.5 Observation table

	Tap water		Pond water
Time	Amount of $NaHCO_3$ added	Number of bubble evolved	Number of bubble evolved
5mins	0.5g	3	17
10mins	1g	6	19
15mins	1.5g	5	16
20mins	2g	5	17
25mins	2.5g	4	15
30mins	3g	3	13

1.6 Results and Discussion

It has shown that the number of bubbles evolved increases in the beginning and then decreased gradually. A mount of light affects the rate of photosynthesis because photosynthesis rate is more when plant is exposed to light than plant kept in the shade. There was evolution of oxygen bubbles from both the set up that is from tap water and pond water with water cress. The number of oxygen bubbles increases in both the set up few minutes after the set up. However, the number of bubbles coming out from the glass tube decreases in both the set up after 20 minutes.

From the experiment, the number of bubbles evolved increases from both type of water in the beginning. It is because in tap water at first the CO_2 content is low. As $NaHCO_3$ is added which supplement CO_2, the bubbles evolution increases. After that, even if the $NaHCO_3$, the number of bubbles evolved keeps on decreasing. It is because, these experiment set up with tap water is kept in shade and even if there is CO_2, there is no light. The light act as limiting factor and thus no photosynthesis can be carried and ultimately no evolution of O_2. On other hand, the pond water set up was kept in sunlight and there was increase in bubble formation after few minutes of set up. The pond water will have more CO_2 than the tap water, hence no $NaHCO_3$ is added. The number of bubbles formation decreases after 20 minutes even though set up was kept in bright sunlight because the concentration of dissolved CO_2 in water reached at low concentration. Here, carbon dioxide act as limiting factor.

1.7 Precautions

1. All the apparatus should be washed properly.
2. select aquatic or semi aquatic plants for experiment.
3. Wilmott's apparatus should be air tight.
4. Number of bubbles evolved during experiment should be recorded carefully with corresponding time.
5. Should add NaHCO3 in tap water not in pond water.

Friday, 15 March 2019

1 Rate of anaerobic respiration

1.1 Aim

To study the rate of anaerobic respiration in different plant parts using Ganong's respirometer.

1.2 Theory

Cellular respiration is the mechanism of breakdown of food materials within the cell to release energy, and the trapping of this energy for the synthesis of ATP. Respiration is a process by which the respiratory substrate is broken down to release energy. Aerobic and anaerobic respirations are the two main operating factors of cell respiration. Aerobic respiration occurs in the presence of oxygen and anaerobic respiration occurs in the absence of oxygen. Glucose is the common respiratory substrate and it is a 6-carbon compound. The substrate is metabolized through glycolysis, TCA cycle, electron transport chain, and oxidative phosphorylation and through these cycles, cells are able to produce and store ATP, and carbon dioxide is produced as a by-product (Patel & Bhardwaj, 2018).

Anaerobic respiration takes place in the absence of oxygen and the substrate is oxidized incompletely with the release of some energy. Anaerobic respiration usually occurs in germinating seeds, in fruits, among microorganisms and in deep-seated tissues of plant and animals (Soni, 2010). Ganong's respirometer is an instrument which is used to measure the respiratory quotient, as respirometer can determine the amount of oxygen absorbed and carbon dioxide released simultaneously. The respirometer consists of bulb with a side tube. The neck of bulb and stopper are provided each with a hole. The side tube is connected to the levelling tube/graduated tube by a rubber tubing.

Respiratory quotient is also known as the respiratory ratio (RQ). It is defined as the volume of carbon dioxide released over the volume of oxygen absorbed during respiration. It is a

dimensionless number used in a calculation for basal metabolic rate when estimated from carbon dioxide production to oxygen absorption. Respiratory quotient is calculated for a particular substrate such as carbohydrates, organic acid, fat, and protein. Carbohydrates are oxidized through aerobic respiration resulting in an equal ratio of carbon dioxide release and oxygen consumption. The RQ value for fat, protein, and anaerobe is 0.7, 0.8, and 0 respectively. The RQ ratio is 0.8, if a mixture of the substrates is consumed (Patel & Bhardwaj, 2018).

Respiratory Quotient (RQ) = Volume CO_2 released/ Volume O_2 absorbed

1.3 Hypothesis

On addition of potassium hydroxide (KOH) the water level in the Ganong's respirometer should increase, indicating that anaerobic respiration is taking place. Rise in the level of water is a direct measurement of the volume of oxygen consumed during respiration by germinating seeds.

1.4 Materials and Method

1. Before the actual experiment, a week before, gram seed were soaked and allowed to germinate.
2. 25 grams pre-soaked seeds which were germinated were then taken in the bulb of Ganong's respirometer and the mouth of the bulb was closed with the stopper.
3. The distilled water was suspended from graduated tube till the level between the two tubes was equal. Initial volume was recorded.
4. The set up was left for one hour and the change in level of the distilled water was noted after one hour.
5. After one hour, few pellets of KOH were added into the bulb where the germinated seeds were kept.
6. The change in level of distilled water in the graduated tube was noted once again immediately after adding KOH.

1.5 Observation table

Initial reading (ml)	After 1 hour (ml)	Addition of KOH after 1 hour (ml) Final reading
13	13.1	13.8

Calculation

Oxygen consumed = final value - initial value
= 13.8 – 13 = 0.8 ml
Carbon dioxide released = Reading after the addition of KOH – Reading after 1 hour
=13.8 – 13.1 = 0.7.ml
Respiratory quotient (RQ) = CO_2 released/O_2 consumed

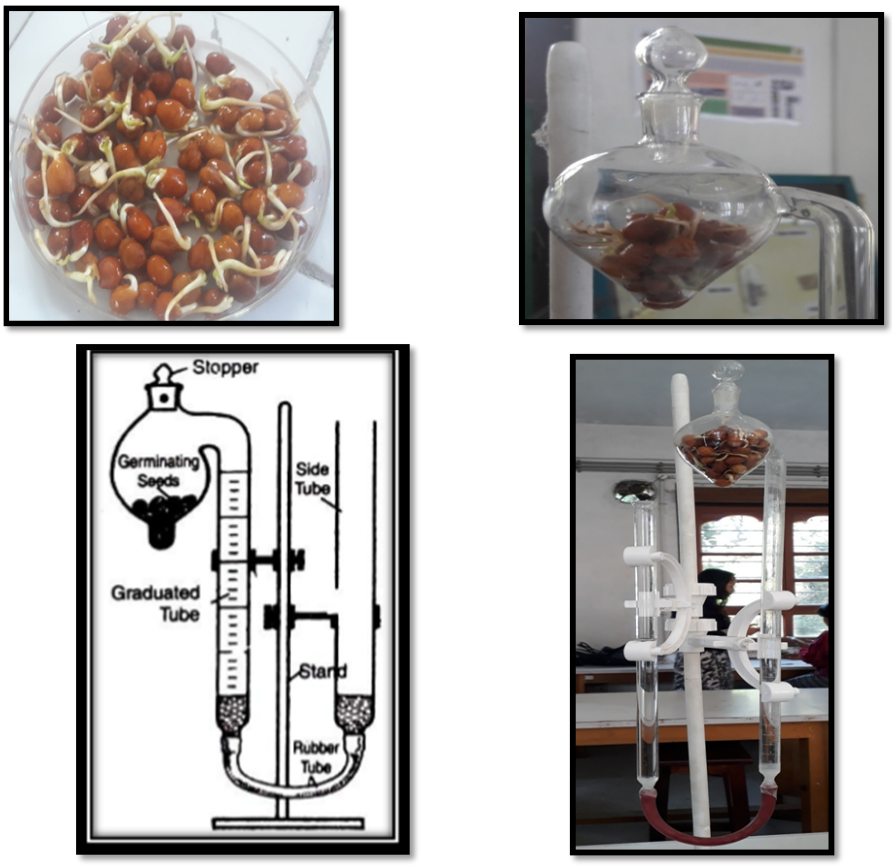


Figure 9: *Ganong's respirometer*

RQ = 0.7/0.8
= 0.875

1.6 Results and Discussion

The respiratory quotient (RQ) of gram is 0.875. When respiration takes place in a closed chamber the Ganong's respirometer bulb, the oxygen is continuously depleted from the atmosphere of the bulb and the carbon dioxide is added. Carbon dioxide is absorbed by the KOH when it is added creating vacuum in the bulb which water level rises to fill up the vacuum. Rise in water level is direct measure of volume of oxygen consumed during respiration by germinating seeds.

1.7 Precautions

1. To obtain result, it is better to use healthy soaked seeds but there should be enough amounts of seeds.
2. Glass stopper should be closed properly to avoid the air getting in and out of the tube.

3. Graduated tube and side tube should be fixed at equal height.
4. Be careful while using Ganong's respirometer to avoid breakage.
5. To avoid leakage at the end of graduated tube and side tube, the rubber tube must be sealed properly.

Friday, 22 March 2019

1 Stomata

1.1 Aim

To estimate stomatal index (SI) and stomatal frequency (SF).

1.2 Theory

Stomata are minute pores found on the epidermis of leaves and young shoots of plant that are used to control exchange of gases. Stomata are mostly present on the surfaces of leaves but they can also be present on inflorescences, fruits, stem, tendrils and other parts of the plant. The pore is surrounded by a pair of specialised 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 vapour through the process called transpiration. Besides this, the exchange of oxygen and carbon dioxide in the leaf also occurs through the stomata Poole et al. (1996).

Distribution of stomata varies between monocots and dicots between plant species and between the underside and top side of the leaves on a plant. Stomata are found more on plant surfaces thriving under higher light, lower atmospheric carbon dioxide and in moist environments. Usually the lower surface of a dicot leaf has a greater number of stomata while in a monocot leaf they are more or less equal on both surfaces. In most of the floating plants, stomata are found only on the upper epidermis (Sack & Buckley, 2016).

1.3 Hypothesis

To study the stomatal distribution on the upper and lower leaf surfaces and to calculate the stomatal index and frequency.

1.4 Materials and Method

1. Take one fresh leaf
2. Take two watch glasses and poured some distilled water into both the watch glass.

3. Split the leaf from the plant obliquely.
4. Take the peel from the upper surface of the leaf using the forceps.
5. Place the peel into the watch glass containing water.
6. Take another peel from the lower surface of the leaf using forceps.
7. Place the peel in the watch glass containing water.
8. Using the dropper, take few drops of safranin solution and poured into the two watch glass.
9. Take two clean glass slides and place the peel leaf on the slides one by one using the brush.
10. Take some glycerine using dropper and put some glycerine on both the slides.
11. Take a cover slip and place it gently on the peel with the help of needle.
12. Take the glass slide and place it under compound microscope.
13. Observe under the microscope.
14. Count the number of stomata in the peels of both upper and lower epidermis of the leaf appearing in the microscope field.

Figure 10: *Agave leaf*

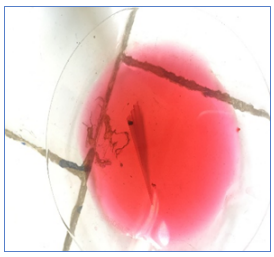


Figure 11: *Peel leaf in safranin solution*

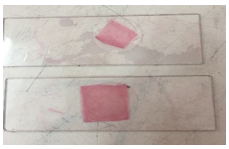


Figure 12: *Leaf peel on slide cover with cover slip.*

Figure 13: *Ocular lens*

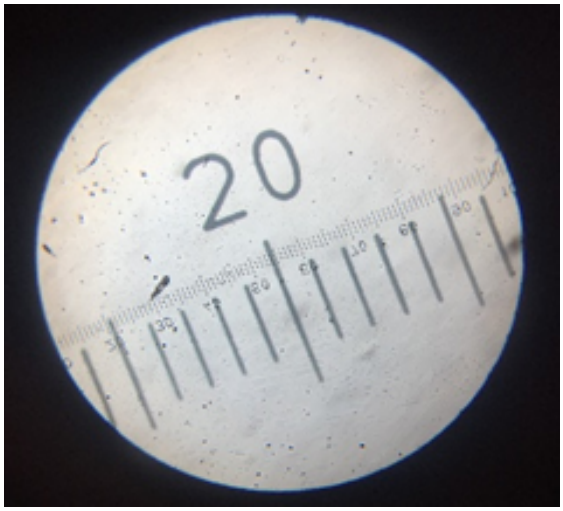


Figure 14: *Adjusted micrometer scales.*

Micrometer calibration

1. Take the ocular micrometer and place inside the compartment of the ocular lens of the compound light microscope.
2. Keep the stage micrometer was on the stage of the compound light microscope.
3. Adjust the microscope until the scales of the micrometers were visible like the figure below
4. Take a clear photograph showing those scales.
5. Take the readings of the first over lapping point to the next over lapping point to find the unit.
6. Then count the point at which the two scales meet to find out the radius.
7. The stage micrometer was then removed to place the glass slides.
8. Count the number of stomata in the peels of both upper and lower epidermis of the leaf appearing in the microscopic field.

Leaf surface	No. of stomata	No. of epidermis cells
Adaxial (upper surface	67	1104
Abaxial (lower surface)	59	912

Calculation: Stomatal index of lower surface:
SI=(S*100)/(S+E) =(59*100)/(59+912) = 6.07% Stomatal index of upper surface SI=(S*100)/(S+E) SI=(67*100)/(67+1104) = 5.72%

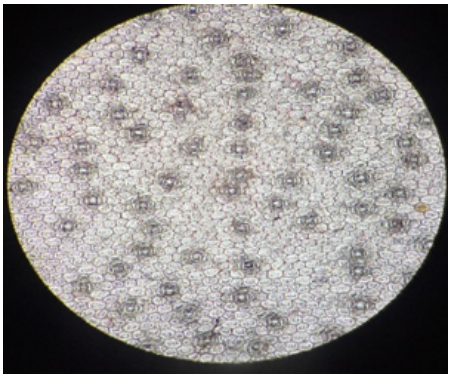


Figure 15: *Micrograph of Adaxial leaf*

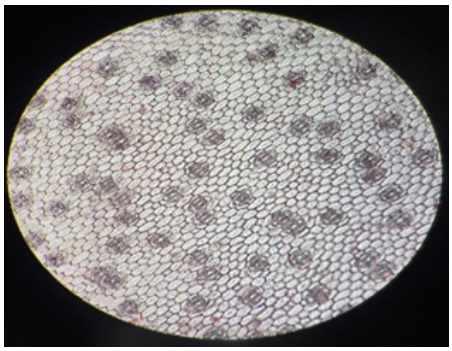


Figure 16: *Micrograph of Abaxial leaf*

Area: Ocular meter reading= 34
Stage meter reading = 10
=34/10
=3.4
Division= 3.4
division= 3.4*0.01
= 0.034 Diameter of the microscopic field = 90
Radius= 45
= 45*0.034
= 1.53
$A=\pi r^2$
$=3.14*(1.53)^2$
$=7.35\mu m^2$

Stomatal frequency of the lower leaf: Frequency = No. of stomata per μm^2
=67/7.35
= 9.11 μm^2

Stomatal frequency of the upper leaf: Frequency= No. of stomata per μm^2
=59/7.35
=8.02 μm^2

1.5 Results and Discussion

The distribution of stomata in the monocot plant (Agave) is more or less equal in both lower and upper surface the leaf. In our experiment, the stomatal index of the lower surface is higher with the value of 6.07% as compared to the upper surface which is 5.72%. Stomatal frequency is higher for the lower surface of the leaf as compared to upper surface of the leaf.

Monocots have stomata on both the upper and lower surfaces of their leaf. In our experiments, we have taken Agave leaf which is monocot plant. Due to this, the distribution of stomata on both side surface will be more or less equal to each other. Sometimes, it shows some variation on its distribution.

This could be because of the plants adaptation to water stressed environment and its response to change in atmospheric levels of carbon dioxide. In our experiment, the distribution of stomata, stomatal index is higher in the lower surface of the leaf as compared to the lower surface of the leaf. However, the stomatal frequency is higher for the upper surface than the lower surface. This minimal variation is due to its adaptation and increase anthropogenic activities in an around the ecosystem.

1.6 Precautions

1. The curling of the peel should be avoided.
2. Always use a brush to transfer the peel from watch glass to the slide.
3. Count the number of stomata accurately.
4. Focus the ocular meter and stage meter exactly to find the exact area.
5. Avoid over staining of the cells.

Friday, 29 March 2019

1 Biochemical changes in protein on freezing

1.1 Aim

To determine the biochemical changes in protein on freezing

1.2 Theory

Freezing is one such stress that has causes a lot of disruption in the normal growth and development of plant and their productivity. Many biochemical changes occur in the plant in response to this stress and degradation of protein is one of them. A plant tends to release series secondary metabolites and most of them are amino acids. Release of amino acids happens as a result of the breakdown of many of its macromolecule, the protein (Hayat et al., 2012).

High salt concentrations as a result of formation of ice during freezing also cause the concentration of the protein to rise in the plants. Degradation of the protein follows due to the creation of the protein instability (Cao et al., 2003).

Plant proteins under goes degradation upon freezing

1.3 Materials and Method

1. Prepare reagents required
2. To prepare concentrated dye reagent:

   Dissolve 100mg of Coomassie Brilliant Blue G-250 in 50 ml of 95% ethanol

   Add 100 ml of conc.ortho phosphoric acid and the solution is then made to a final volume of 200 ml with distilled water.
3. To prepare diluted dye reagent:

   Mix 1 volume of concentrated dye solution with 4 volumes of distilled water.

   Filter the solution with Whatman no. 1 filter paper if any precipitate occurs.
4. 10 mg/10 ml BSA stock is prepared
5. To extract protein from the leaf:

   Take 200 mg of fresh leaf sample

   The leaf sample is crushed with 5-10 ml of distilled-water using the pestle and mortar

   The solution is filtered and centrifuged
6. Pipette out 10 ml of diluted BSA solutions in a series of testtubes
7. Pipette out 10ml of diluted sample extract (2 ml sample + 8 ml water) in another tube. A tube with 10ml of water serves as blank
8. Add 5ml diluted reagent to each tube
9. Vortex well
10. Allow the color to develop for 5-30 minutes (the red dye turns to blue when it binds with protein)
11. Measure absorbance at 595 nm.

1.4 Observation table

Concentration (mM)	Absorbance of BSA (OD)	Absorbance of Before freezing (Au)	Absorbance of After freezing (OD)
10^{-5}	-0.085	0.088	0.078
10^{-4}	0.0146	0.143	0.105
10^{-3}	0.059	0.159	0.126
10^{-2}	0.256	0.190	0.133
10^{-1}	0.913	0.273	0.193

%

The protein content decreases on freezing and it also decreases with the increase in the concentration of the sample extract.

The Bradford Coomassie brilliant blue G-250 protein-binding dye exists in cationic, neutral, and anionic forms. The anion form binds with the protein. The dye binding requires a macromolecular form with certain reactive functional groups. The

interactions are mainly with arginine. Other primary amino groups such as the other basic (His, Lys) and aromatic residues (Try, Tyr, and Phe) give slight responses. The binding behavior is due to Van der Waals forces and hydrophobic interactions (Compton & Jones, 1985).

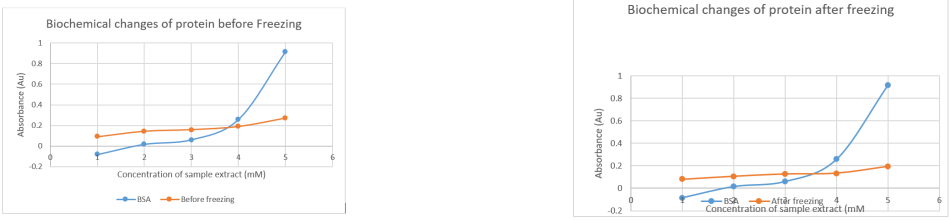


Figure 17: *Graph showing the biochemical changes of protein on freezing.*

Freezing stress usually causes structural and functional cellular damages and intracellular dehydration. Protein content of the leaf, on freezing, decreases due to increased protein degradation (Shin et al., 2018).

1.5 Precautions

1. Measurement of chemicals must be accurate during the preparation of BSA
2. Serial dilution must be done without mixing the different concentrations.
3. Leaves must be freezed before making an extract.

Friday, 5 April 2019

1 Effects of saline stress on the germinability of seeds

1.1 Aim

To study about the effects of saline stress on the germinability of seeds (chili seeds).

1.2 Theory

Salinity is one of the major environmental factors that diminish crop productivity and threaten the global balance of food. In arid and semi-arid regions of the world, some of the most serious soil salinity problems occur. Out these regions, salinity also affects agriculture in coastal areas and in areas affected by irrigation water of low quality. Germination and early stages of development should be confronted by higher salinity levels than the later stages, as the grown plants are more vigorous,

and because germination and early seedlings growth take place in surface soil where there is a high salt accumulation due to evaporation and climbing of water by capillarity. One of the most detrimental effects of salinity is the accumulation of Na^+ and Cl^- ions in plant tissues subjected to NaCl soils (El Goumi et al., 2014).
Salinity is one of the environmental factors that have a critical impact on halophyte seed germination and plant establishment. Salinity is one of the biggest abiotic stresses in arid and semi-arid regions, affecting 7% of the world's land area. Salinity reduces plants ' ability to absorb water, resulting in a metabolic effect that reduces plant growth. By upsetting plant water and nutritional balance, it can reduce crop yield. Hyper-osmotic shock and ionic imbalance are the deleterious consequences of high salt concentrations in the outer solution of plant cells (Wu et al., 2015). Salinity creates an external osmotic potential that limits seed water absorption, or sodium and chloride ions may accumulate in the germinating seed, leading to a toxic effect (Bouaziz et al., 1990).

1.3 Hypothesis

With increase in the salt concentration, the germination of the seed is reduced or The rate of germination of seed decreases as salinity increases and increases with decrease in salinity.

1.4 Materials and Method

Preparation of solutions for saline:

1. Various concentrations of saline solutions have been prepared (10%, 15%, 20% and 25%).
2. By measuring 10g of sodium chloride (NaCl) and dissolving it in 80 ml of distilled water, 10% of the saline solution was prepared.
3. By adding distilled water, the final volume was made to 100ml.
4. By measuring 15g, 20g and 25g of NaCl in a digital weighing balance and dissolving it in 80 ml of distilled water, 15%, 20% and 25% of the saline solution were prepared.
5. By adding distilled water, the final volume of all the different concentrations (15%, 20%, and 25%) of saline solution was made to 100ml.

Seed sterilization (Gram seed) : Using 0.02% mercuric chloride ($HgCl_2$), the gram seeds were sterilized.

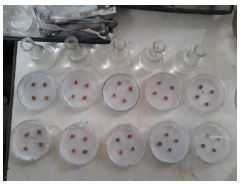



Figure 18: *Germination of gram seeds observed in the control set up.*

Procedure -

1. Fifteen petridish sets have been washed and kept ready for the experiment.
2. Blotting paper was circularly cut and placed inside the petridish.
3. Fifteen chili seeds were placed inside each petridish on the blotting paper.
4. Each petridish was marked as a replica (R1, R2 and R3) along with the saline solution concentration (10%, 15%, 20% and 25%).
5. The establishment of controls was also prepared.
6. The setup of the control was marked as control and three replicas were produced.
7. Different saline solution concentrations have been added in each set.
8. Three replicas of the plates containing the same number of seeds were made for each concentration and the same amount of saline solution was added.
9. Only water was added to the controlled setup.
10. The petridish's lid was closed and the setup was observed.
11. Observation was done for a week after every two days.
12. The number of germinating seeds has been recorded.

1.5 Observation table

Replica	Control	10 mM	15 mM	15 mM	15 mM
R1	5	1	2	0	0
R2	4	2	0	1	0
R3	4	0	0	0	0

It is clear from the observation table that the seed mostly germinated in the set-up of the control. In the different concentration of saline solution, the seeds did not germinate at all or only few germinated. In the controlled setup, five seeds in the R1, four seeds in R2 and four seeds in R3 germinated.

The seeds in the controlled setup germinated well as in that experimental setup the amount of salt was minimal. The chili seeds were unable to germinate in the different concentration of saline solution or only few germinated as the quantity of salt exceeded the optimum level and may have caused the seeds to suffer from salinity stress. The increase in salinity reduces the germination rate of the seed. Seeds did not germinate in the various concentrations of saline solution because high quantities of salts resulted in the seeds reducing water intake.

1.6 Precautions

1. Before use, the seeds should be sterilized.
2. There was a high concentration of different concentration of saline solution that was prepared.
3. To compare the results, replicas should be done.
4. In each petridish the same quantity of saline solution should be added.
5. Blotting paper should be cut to suitable size.

Friday, 12 April 2019

1 Buffering capacity

1.1 Aim

Preparation of buffers (Phosphate), study of buffering capacity of prepared buffer and of plant juice.

1.2 Theory

The biological system has the capacity to maintain the acid-base balance to a specific pH. The buffering capacity of a solution assists in day to day activities of an individual. A buffer system is a solution that resists a change in pH when acids or bases are added. The buffering capacity means the amount of H+ required until the pH value of a solution changes by the unit 1.

Buffers are solutions that contains a weak or medium strong acid (base) and the salt of the corresponding base (acid) in about similar concentrations (Proksch, 2018). The change in pH is determined by the metabolic processes of a plant cells (Scholz & Reck, 1977).

The hydrogen concentration in vitro is stabilized by adding a suitable buffer to the medium. A buffer keeps the pH of a solution constant by taking up protons that are released during reactions, or by releasing protons when they are consumed by

reactions. A buffer solution is a solution that resists changes in pH either when diluted or when limited amounts of acid or base are added to it. Such a solution can be prepared by combining a weak acid and its salt with a strong base or analogously, a weak base and its salt with a strong acid (Urbansky & Schock, 2000).

Phosphate is originated from phosphoric acids having a good buffering capacity. The degree of dissociation is depended on its pH. This is depended on the physio-chemical factors such as the ionic strength and mineral environment(Salaün et al., 2005).

1.3 Hypothesis

Plant extract with low buffering capacity will show rapid falls in the pH and gradual changes in the pH for the high buffering capacity on adding the HCl or KOH.

1.4 Materials and Method

1. A tomato was taken, washed and cut it into small pieces using a knife.
2. Using the mortar and pistil, the cut pieces was made into pulps.
3. Then a cotton cloth was taken and the pulp was squeezed through the cotton cloth.
4. Using a measuring cylinder, 10 ml of juice extraction was measured and poured in two beakers.
5. Then 0.5 N KOH and 0.5 N HCl was prepared.
6. For the preparation of 0.5 N HCl:
   a) 25 ml of distilled water was measured and poured in a conical flask.
   b) 0.45 ml of HCl was measured and mixed with the 25ml water.
7. For the preparation of 0.5 N KOH:
   a) 25 ml of water was measured and poured in a conical flask.
   b) 0.7 gram of HCl was weighed and mixed with 25ml water.
8. Two burettes was taken and filled with KOH and HCl respectively.
9. The conical flask with the tomato juice was titrated with the acid and base respectively.
10. The procedure was repeated by adding 1ml of HCl and KOH, till the pH of the base reached 14 and pH of the acid reach 2.

11. pH meter was calibrated with the tap water at a pH of 7.
12. pH meter is then immersed into the tomato extract and the same process was repeated till it reached the desire value.

Figure 19: *Step 1. Left: Tomatoes were grinded. Right: Extract of tomatoes were kept in two beakers*

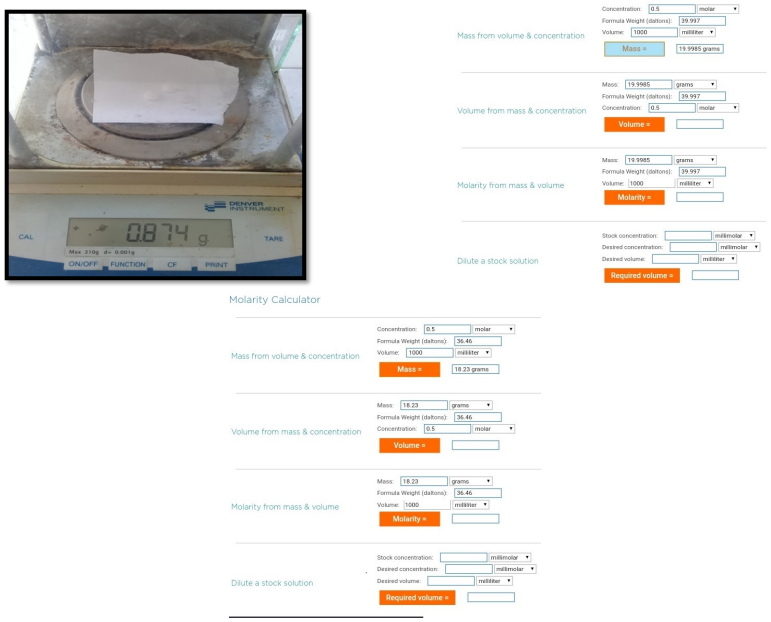


Figure 20: *Step 3: Titration of both the extract were done.*

1.5 Observation table

Volumn of HCl	0 ml	2 ml	3 ml	4 ml	5 ml	6 ml	7 ml	8 ml	9 ml	10 ml
pH change on adding HCl	4.6	4.3	3.8	3.6	3.2	2.8	2.4	2.2	2	
pH change on adding KOH	4.6	11.1	11.9	12.2	12.4	12.5	12.8	13.1	13.5	14

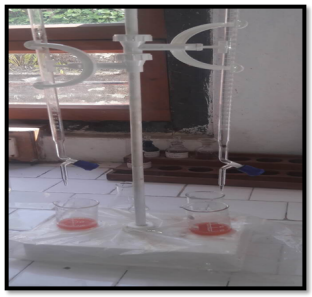
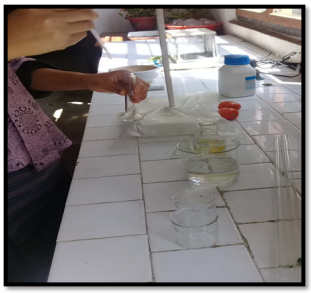



Figure 21: *Titration was carried out for both the extract*

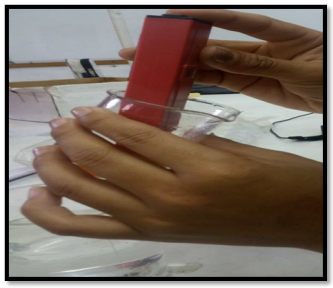



Figure 22: *KOH and HCl were added till the pH reached 14 and 2 respectively*

Buffers keep the solution neutral despite the acidic or basic environment because of its nature of either being acid or base. The pH of the extract depends on the addition of acid or base. If the change in pH of the extract is rapid it is considered as of having low buffering capacity and if the pH changes gradually on addition of acid or base then the extract is considered of having high buffering capacity.

The experiment carried shows that the increase in the concentration of the HCl decreases the pH value thereby making the tomato juice more acidic. On other hand the pH value increases with the increase in concentration of KOH, which shows that tomato juice becomes more basic.

The pH of the extract shows a rapid increase and decreases with the addition of 1ml of KOH and HCl respectively. However, after sometime, the pH increases and decreases gradually. This gradual change in the pH of the tomato extract shows the capacity to resist change in the concentration and thus tomato can be classified as having a high buffering capacity.

1.6 Precautions

1. The tomato should be finely grinded for the accurate result.
2. The measurement of the chemicals should be precise for minimizing error in the result.
3. pH meter should be calibrated with the distilled water before starting the experiment.

4. pH meter should be kept for sometimes in the extract to obtain the accurate pH.

Friday, 10 May 2019

1 Absorption spectrum

1.1 Aim

TTo study the absorption spectrum of chlorophyll pigment.

1.2 Theory

The most abundant pigment in plants is chlorophyll, which gives plants their green color. Light is essential for chlorophyll formation in higher plants, and it is likely that this process involves the building up of a light-sensitive precursor in the dark, and the subsequent formation of chlorophyll upon radiation (Frank, 1946).

Higher plants contain primarily two types of chlorophyll namely chlorophyll a and chlorophyll b. Chlorophyll a is bluish green while b is yellow in color. The contents of the chlorophyll a are usually three times higher than the b in leaf tissue. Chlorophylls are insoluble in water but soluble in organic solvents (Palta, 1990).

Each plant pigment is characterized by its specific absorption spectrum. Relative absorbance of various wavelength by pigment can measure with a spectrophotometer. Chlorophyll a and b absorb both blue light (400 – 500 nm) and red light (600-700nm). The absorption spectra of the same pigment may vary to some extend depending upon the solvent that is used for the extraction of pigment (Cinque et al., 2000).

1.3 Hypothesis

The highest peaks represent colors that chlorophyll absorbs the most and lowest peaks represent color that chlorophyll reflected.

1.4 Materials and Method

1. Preparation of the filtrate
2. 10 ml of 80% acetone was prepared.
3. 0.5 mg of fresh leaf material was accurately weighed after removing midribs.
4. The leaf material (Fagopyrum) was grounded in a mortar and pestle using 5 ml of 80% acetone.

5. The homogenate is filtered using Whatman paper 1 and filtrate collected in a 25 ml volumetric flask (covered with aluminium foil to protect pigments from photo-oxidation).
6. The homogenate was washed 2-3 times with (5ml of 80% acetone each time) and final volume of filtrate made to 15 ml.
7. The optical density (OD) of the chlorophyll extract is measured from 400-700 nm wavelengths using Spectro-106.
8. 80 % of acetone is used as a blank.

.

1. The spectrophotometer was switched on.
   a) Then it was kept on for 20 minutes to warm up.
   b) The wave length was set (663nm and 645nm) using the WI knob.
   c) For the calibration:
   d) Pressed the REF button
   e) The display showed ZERO
   f) Selected the dark filter
   g) Pressed the REF button
   h) The display showed CAL
   i) Then waited for few seconds
   j) Inserted the blank that was the 80% acetone for reference
   k) Selected the filter for the selected WI
   l) Pressed the REF button
   m) The display showed CAL
   n) Then waited for few seconds
   o) The reference was calibrated to 100% T
   p) Pressed the mode switch for ABS
   q) The display showed 0.000
   r) Inserted the sample
   s) Closed the lid and took the reading
2. This was repeated for using the wavelength 400nm-700nm with the interval of 5nm.

1.5 Results and Discussion

Sl.no.	Wavelength	Absorbent	31	550	
1	400	2.421	32	555	0
2	405	2.41	33	560	0
3	410	2.386	34	565	0
4	415	2.38	35	570	0
5	420	2.354	36	575	0
6	425	2.336	37	580	0
7	430	2.324	38	585	0
8	435	2.291	39	590	0
9	440	2.27	40	595	0
10	445	2.24	41	600	0.002
11	450	2.195	42	605	0.01
12	455	2.15	43	610	0.101
13	460	2.092	44	615	0.179
14	465	2.047	45	620	0.157
15	470	1.987	46	625	0111
16	475	1.873	47	630	1.029
17	480	1.612	48	635	0.959
18	485	0.713	49	640	1.008
19	490	0.285	50	645	1.212
20	495	0.035	51	650	1.56
21	500	0	52	655	2.097
22	505	0	53	660	2.289
23	510	0	54	665	2.277
24	515	0	55	670	2.151
25	520	0	56	675	1.895
26	525	0	57	680	1.207
27	530	0	58	685	0.654
28	535	0	59	690	0.364
29	540	0	60	695	0.141
30	545	0	61	700	0.013

Color	Wavelength	% of light absorbed	% of light reflected
Voilet	380-450 nm	43.49	56.51
Indigo	425-450 nm	23.19	76.81
Blue	450-500 nm	27.26	72.74
Green	500-565 nm	0.059	99.94
yellow	565-590 nm	0	100
Orange	590-625	0.95	99.05
Red	625-740 nm	32.21	67.79

It is evident that the absorption spectrum of chlorophyll pigment is higher at wavelengths 450-475nm and 650-675nm. The absorption is higher at violet color (380-450nm), that is 43.48% and followed by red(625-740nm), blue(450-500nm), indigo(425-450nm), orange(590-625nm), green(500-625nm) and yellow(565-690nm) with 32.2%, 27.2%, 23.1%, 0.9% and 0.059 respectively.

When the light strikes on leaves of the plant, the chlorophyll pigments absorb the light. The absorption of light is proportional to the amount of chlorophyll pigments present in the leaves. It is evident from the experiment that the chlorophyll extract of *Fagopyrum dibotrys* leaves shows higher absorption at the range of wavelength 450-475nm and 650-675nm because

the primary photosynthetic pigment absorbed (chlorophyll a) mainly the wavelength between 450-475nm and accessory pigment (chlorophyll b) prefer at absorption at the wavelength ranges between 650-675nm. The absorption peak sharply drops below 680nm wavelength in the green plants and its effects is known as red drop. The primary and secondary pigment appears green in color since light wavelength ranges between 500-600 are least absorbed by the green plants and they reflect the green color of the spectrum.

1.6 Precautions

1. Preparation of acetone concentration should be taken care.
2. Filtration should be done with Whatman paper
3. The filtration should be properly covered by aluminum so that the photo oxidation does not take place before the measurement.
4. The cuvette should be handled with care and should be cleaned regularly.
5. The transparent side of the cuvette should be faced to the light beam.

Friday, 17 May 2019

1 Chlorophyll quantification

1.1 Aim

Spectrophotometric quantitation of chlorphyll pigment "a" and "b".

1.2 Theory

Chlorophyll is a green photosynthetic pigment found in plants, algae and cynobacteria. Cholorophyll molecules are specifically arranged photosystem,which are embedded in the thalakoid membrane of choloroplasts. Chlorophyll "a" is the most important light absorbing pigment in the plants. Its absorbs all the light pigments except the green light.The other pigments like chlorophyll b, carotenoids and xanthophyll are the accesory pigments (Jakab-Ilyefalvi & Pamfil, 2008).

Chlorophyll does not absorbs all the wavelengths of visible light equally. The chlorophyll "a" absorbs light maximum with wavelength of 430 and 662 nm.Plants do not depends only on chlorophyll "a" in their light harvesting machinery but also have other pigments(accessory pigments) which absorbs light of different wavelengths. Various chlorophyll and accessory

pigments have characteristics absorption spectra. The action spectra of photosynthesis relates to the relative electron exciting effectiveness of different wavelength of light.

Spectrophotometry is the classical method of determining the quantity of chlorophyll in plants,A spectrophotometer is an instrument that measure the ability of an object to absorbs specific wavelength of light. When the amount of light absorbed versus wavelength is plotted, it is referred to as an absorption spectrum. By measuring the absorption of light in the red and far red region, it is possible to estimate the concentration of chlorophyll within a leaf. The pattern of absorption of light by chlorophyll a and b is shown in the figure 1 (Porra et al., 1989).

Working with spectrophotometer: In a spectrophotometer, a beam of light is passed through a prism or filter of a given colour, which absorbs part of the colour. The remainder is transmitted through a solution, strikes a photoelectric tube, and is converted to electricity, which is then measured by the spectrophotometer. The absorbed light at any wavelength is the total light minus the amount transmitted (in the experiment, the sample absorbs green light).

1.3 Hypothesis

Cholorophyll does not absorbs all the wavelength of visible light equally. The cholorophyll "a" absorbs maximum light with wavelength of 430 and 662nm.

1.4 Materials and Method

1. 0.5g of leaf of Rumex nepalensis was weighed in weighing machine. While measuring the leaf, vein and midrib were removed.
2. 15ml of 80% acetone solution was prepared by mixing 8ml of acetone in 2ml of distilled water.
3. 5ml of 80% acetone was added to the pestle along with the 1g of leaves and it was crushed. It was grinded throughly until the last bit of leaves were visible.
4. It was then filtered using filter paper and funnel.
5. The residue left on the funnel was again ground with 5ml of 80% acetone.
6. The residue left on the filter paper was again grinded and filtered to obtain enough filtered.
7. Firstly, 10ml of 80% acetone was taken as reference or blank.
8. Next,O.D (optical density) reading of the chlorophyll extract was recorded at different wavelength at 5nm interval ranging between 340- 750 nm.

9. For cholorophyll a and b O.D at 645nm and 663nm was recorded.
10. The amount of cholorophyll present in the extract was measured.

Calculated according to the equation given below
Chlorophyll a = 12.7 (663nm) -2.69 (645nm)
Chlorophyll b = 22.9 (645nm) - 4.68 (663nm)
Total chlorophyll =20.2 (645nm) + 8.02 (663nm)
Calculation (Quantification of Chlorophyll a and b)

- Chlorophyll a Chlorophyll a = 12.7(A663) - 2.69(A645) * V/1000 * W
  = 12.7(2.288) – 2.69(1.212) * 10/1000 * 0.5
  = 0.5154 g/g^{fw}
- Chlorophyll b Chlorophyll b= 22.9(A645) – 4.68(A663) * V/1000 *W
  =22.9(1.212) – 4.68(2.288) * 10/1000 * 0.5
  =0.3416 g/g^{fw}
- Total chlorophyll (a+b) = 20.2(A645) + 8.02 (A663) * V/1000* W
  = 20.2(1.212) + 8.02(2.288) * 10/1000 *0.5
  = 0.856 g/g^{fw}

1.5 Results and Discussion

When the light fall on the leaf surface, the chlorophyll pigments absorbs the light. The absorption of light is proportional to the amount of light chlorophyll pigments present in the leaves. From the experiments, we found out that the total amount of chlorophyll present in 0.5 g of leaves of *Rumex nepalensis* is 0.856 g/gm^{fw} of which 0.5154 g/g^{fw} is chlorophyll a and 0.3416 g/g^{fw} is chlorophyll b.

1.6 Precautions

1. Right amount of leaf should be used to get a proper absorption spectrum.
2. The leaf veins should be removed.
3. The spectrophotometer should be handled with care.
4. The 80 % acetone should be prepared properly.

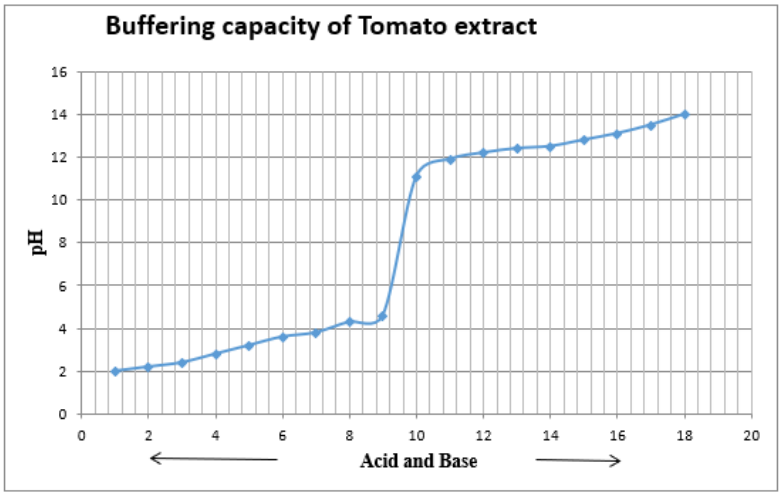
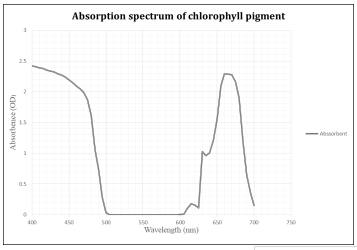
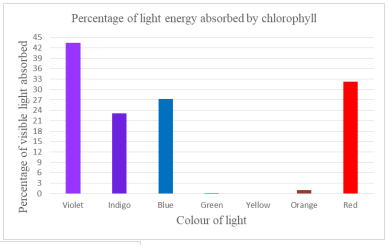


Figure 23: *Graph of bufffeing capacity of tomato.*

Figure 24: *Spectrophotometric estimation*





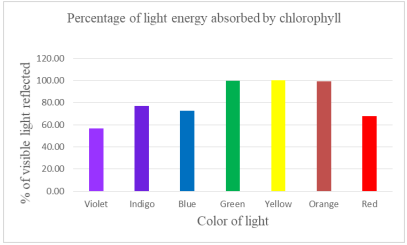


Figure 25: *Absorption spectrum*

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