**Membrane Function Experiment on Measurement of Water and Osmotic Potential Alongside Other Movements**

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**Introduction:**

The experiment was performed by BIOL 120L students in the biology lab on March 9th, 2023. This experiment aimed to understand further how membranes control water and solute movement, how carbon dioxide can affect the opening and closure of stomata, what water potential and osmotic potential are and how they affect diffusion and osmosis process, and how turgor pressure to drive the plant to move. In general, we are studying the regulation of passages of water and gas in plants macroscopically and microscopically and their effects. During the experiment, we used standard methods such as observation and experiment with control groups and statistical conclusions on data collected to demonstrate and discuss results.

The first part of this Lab is to observe the stomata aperture under different conditions. Stomata are numerous tiny pores on the epidermis of plants. They are usually located on the lower side of the leaves' surface. They are surrounded by guard cells that can control their opening and close through turgor pressure. Stomata play an important role in gas exchange and water adjustment inside the plant. When stomata are open, water will be evaporated from the plants, but the plant will also take gas for its needs, such as carbon dioxide, to maintain essential reactions such as photosynthesis. And when stomata close, no gas and water will enter or exit the plants (Bidlack et al., 2020). In the experiment, we will observe the status of stomata when the plant leaves are placed under different CO2 environments, discuss what factors can affect them, and understand why carbon dioxide conditions can affect them.

While the first part demonstrates the gas exchange through the stomata of plants with the outside atmosphere, part B of the experiment requires us to study how membrane manages to regulate the passage of different materials in and out of cells or organelles. We will use temperature to put abiotic stresses on living beet cells in water and use spectrophotometers to examine the liquid. In beet cells, there is a pigment, usually in a reddish violet color, called Betacyanin. Under normal conditions, this pigment is in the central vacuole (Sadowska-Bartosz & Bartosz, 2021). Therefore, there are two different membrane layers to protect Betacyanin inside cells: the vacuolar and plasma membranes. Membranes of cells and organelles can be seen as a protection of cell contents and are semipermeable, meaning different substances will diffuse through them at different speeds (Bidlack et al., 2020). In our experiment, we will have a control under the normal condition without treatment and two groups under extreme conditions. If the membrane can protect cells and function adequately, we should expect faint, or no color in the solution representing that only a few pigments are leaked out of cells. Otherwise, the membrane might be broken, and thus we will observe the dark color. We will use a spectrophotometer to measure the color to tell if it is faint or dark. We let a light with a fixed wavelength go through the solution and measure an index representing the absorbance of that light. Usually, a higher absorbance rate means darker color.

In part C, we will use the plasmolytic method to measure the osmotic potential of asparagus fern parenchyma cells. Osmosis is when a solvent moves spontaneously through a semipermeable membrane from a low-concentrated solution to a highly concentrated one. In our scenario, where we mainly discuss plants, the solvent is water. We use osmotic potential (ψπ)to describe how likely water will move under the effect of different solute concentrations. Osmotic potential is always negative; a lower concentration solution is considered to have higher osmotic potential, and pure water has zero osmotic potential. Another important potential, turgor/pressure, needs to be considered. Turgor potential, also called turgor pressure, is always a positive value and is a pressure against the cell wall; as water enters the central vacuole, it becomes bigger but will be restricted physically by the size of the cell wall. Generally, we use water potential to describe the intention of water movement through the osmosis process, which is measured by osmotic potential plus pressure potential. Water will flow from regions with higher water potential to regions with lower water potential (Bidlack et al., 2020). In this part, we use the plasmolytic method, which means that we will manipulate the solution concentration to achieve the state that the pressure potential of the plant cells roughly equals zero. Thus, we can conclude that the solution's water potential is the same as the osmotic potential of the cells (Knudson & Ginsburg, 1921). We know that we achieved such a state by an estimate that roughly half of the cells are plasmolyzed, meaning that cells have shrunk membranes, representing that they lose water.

In part D, we will measure the water potential of potato tissues by measuring the weight. We put potato tissues in different solutions. We know the potato might lose or gain water due to osmosis because the solutions all have different potentials that could be higher or lower than potato tissues. If we can find a solution that does not cause weight change in potato tissues, we know that passage of water reaches equilibrium in this case. By definition, the solution has the same water potential as potato tissues. Notice that there is no control in parts C and D.

Finally, in part E, we will observe the turgor movement in plants. Turgid means cells gain water in the vacuole, which can expand in size to support the cell structure by turgid pressure. The purpose is to observe the reaction of Mimosa in response to outside stimulations and understand how turgor pressure controls the process. There is no control in this part.

**Materials and Methods:**

Please refer to BIOL 120L Lab Manual, section 103, winter 2023, laboratory #7, pages 51 to 59. (Department of Biology, 2023) (pages 51-52 for experiment A, but we are not provided with the leaves grown under normal conditions to observe; pages 52-53 for experiment B; pages 55-56 for experiment C, except steps 1-6 were performed in advance by TAs thus skipped; page 56-57 for experiment D, but we record our group data on paper and TA collected data from every group which will be used in the report; page 59 for experiment E)

**Results:**

*Part A: Control of Stomatal Aperture*

**Text 1**: Stomata Aperture

We checked the stomata aperture size of Tradescantia leaves under two different atmospheres with different carbon dioxide concentrations. We observed that the stomata are closed for leaves that are in an environment with a high concentration of carbon dioxide for ten or more minutes, and the stomata open large for leaves that are in the sealed environment for two or more hours where carbon dioxide is finally used up to the concentration of zero. As a control group, we should have a sample for leaves under normal daylight conditions, but it is not provided.

*Part B: Membrane Function*

**Table 1:** The Absorbance at 525 nm of Betacyanin of Beet Cells under Different Temperature Conditions

|  |  |  |  |
| --- | --- | --- | --- |
| **Temperature** | 0 | 22(room temp) | 70 |
| **A525** | 0.052 | 0.092 | 0.985 |

We blade three beet bore samples, put them in different tubes with distilled water, and treated them with different temperature conditions for 30 minutes. The solution in the tubes will have colors, and we take samples and use a spectrophotometer to measure the transparency, thus as an indicator of the shade of the color. The temperature shown in Table 1 is Celsius, indicating the temperature condition. A­525 is the absorbance at 525 nm readings from spectrophotometers; higher numbers mean lower transparency. The second column in the table is the control group when the test tube is put at room temperature because it is under no treatment.

*Part C: Measurement of Osmotic Potential*

**Figure 1:** Percentage of Plasmolyzed Cells in 50 Samples Found under Different Concentration Levels of Mannitol Solution to Measure Osmotic Potential

We take a solution of parenchyma cells and use them to create solutions of parenchyma cells with different mannitol concentrations. We let it rest for 30 minutes, then took a sample drop on a slide and observed it under a compound scope. We randomly sampled 50 cells from the scope view and counted the number of plasmolyzed cells, and the result was used to estimate the total plasmolysis degree. We do not have a specific group for control purposes, but these groups work together and show the result in the cross-reference. Figure 1 demonstrates the roughly linear relationship between the percentage of plasmolysis and mannitol concentration. From the result, we can see that the plasmolysis rate increases with the mannitol concentration.

*Part D: Measurement of Water Potential*

**Figure 2**: Weight Gained by Potato after Treatment of Mannitol Solution with Different Concentrations to Measure Water Potential.

We cut and weighed potato bores, then put them in test tubes immersed with mannitol solutions with different concentrations. After 90 minutes, we take the bores out and measure the weight. Then we calculate the weight difference before and after. A positive value means potato bores gained weight, and a negative value means potato bores lost weight. The data point forms roughly a linear relationship between solution concentration and the gained weight. Soaking potato bores with higher concentrations will let the potato bore gain less weight or even lose weight. We estimate the relationship using linear regression, and the cross-over point means that the potato does not change weight after soaking in mannitol. There is no control in this experiment.

*Part E: Turgor Movement*

**Text 2**: When we touch a leaf of the Mimosa plant, leaflets will fold to close reasonably quickly. We also observed a dry Mimosa plant, and its leaflets are all closed almost the same way the normal Mimosa plant reacts to our touch.

**Discussion:**

*Part A: Control of Stomatal Aperture*

Stomata are responsible for gas exchange between plants and the external environments and water transpiration for plants. The plants must maintain a balance to achieve an equilibrium condition that they do not lose too much water and get enough carbon dioxide since both are essential for photosynthesis (Bidlack et al., 2020). Therefore, the plant will sense its intercellular concentration of carbon dioxide and then use guard cells to control the opening or closing of stomata. When the plant is placed in a CO2­-rich environment for 10 minutes, it will keep taking CO2 inside until its intercellular concentration reaches a threshold enough for photosynthesis. Then it will close the stomata to prevent water loss, as Text 1 indicates. When the plant is grown in a CO2-free atmosphere, the plant will run out of CO2 pertained inside, and the intercellular concentration will become low; thus plant will open stomata, as Text 1 mentioned, seeking for more CO2 to maintain photosynthesis in the price of water loss, since CO2 is an essential material for photosynthesis. Both (Kim et al., 2010) and (Xu et al., 2016) have shown that a higher concentration of carbon dioxide can signal the guard cell to activate the outward movement of potassium ions from guard cells to reduce turgor pressure to cause stomata to close. Since we do not have control, we could not tell the stomata aperture under normal conditions. However, we should expect the stomata will be open under normal daylight conditions for plants to preserve photosynthesis.

*Part B: Membrane Function*

According to Table 1, the test tube treated under room temperature is the control among all three groups and has a reading of around 0.092. The sample solution from the test tube under 70 Celsius degree had an extensive reading from the spectrophotometer, 0.985, indicating that the light is mainly absorbed, and we can tell that the color is much darker than the one from the control. This shows that Betacyanin inside cells is leaked a lot, and either the membrane has increased its permeability to allow more pigments through under the effect of high temperature, or the high temperature has destroyed the membrane completely. On the other hand, the test tube under a 0-degree environment had a lower reading with a fainter color than the control, and less pigment Betacyanin went through the cell membranes. This indicates that lower temperatures can reduce the permeability of membranes. (Quinn, 1988) has shown that higher temperature makes membranes more permeable, and lower temperature makes membranes less permeable, which matches our observation and conclusion. Moreover, since the 70-degree treated sample has a much higher reading (almost ten times) than the control, we can conclude that this temperature is most damaging to the membranes and has broken them down.

*Part C: Plasmolytic Method*

There is no control in this part. Figure 1 indicates a linear relationship between mannitol concentrations and the percentage of plasmolysis. When treated with 0.2 M concentration, almost all parenchyma cells are turgid, and increasing concentration will result in more plasmolyzed cells. 0.4 M concentration results in about 20%, 0.6 M results in about 44%, and 0.8M has the highest plasmolysis rate of around 78%. The result is only a rough estimation because we only sampled a small number of cells from the view, which is highly dependent on the drop we get from the test tube that might not be swirled thoroughly and which part of the solution we observe in the compound scope view, thus the curve is not a precise linear. Nevertheless, we find the estimated line and set y to 50% to solve x, which gives that . According to (Knudson & Ginsburg, 1921), a solution with this concentration should have its water potential the same value as the osmotic potential of the parenchyma cells. We retrieve the osmotic potential from the graph: -600 mOsm, which is about , and this is the average osmotic potential.

*Part D: Gravimetric Method*

Referring to the data points from Figure 2, the weight gained by a potato pore is roughly in a linear relationship with the concentration of mannitol solutions. Potato pores soaked in 0.05M mannitol gained the most weight among the seven groups, and as the concentration increases to 0.1M, 0.15M, and 0.2M, the weight gained by potato pores gradually reduces. Those mannitol solutions have relatively lower concentrations and thus higher osmotic potential, and potato has a fixed water potential that we want to measure, and it is lower than that of solutions so that water will flow from the solution to cells (Bidlack et al., 2020); thus, they will gain weight. It is harder for water to move into cells as the osmotic potentials of solutions decrease since the water potential difference gets smaller and smaller. At 0.3M mannitol, the potato pore roughly remains the same weight, meaning the water flow is in equilibrium, and the osmotic potentials of solutions and potato cells are roughly equal. Then as the concentration grows to 0.4M and 0.5M, potato pores start losing weight by losing water since now the mannitol solutions have a lower water potential than tissue cells; thus, water flows outwards cells. 0.5M mannitol causes more weight loss than 0.4M mannitol because the former has a lower water potential and a more significant potential difference between solutions and cells; thus, water is more likely to move outwards cells (Bidlack et al., 2020). From Figure 2, we can calculate the concentration causing no weight loss or gain in tissue by . The water potential of mannitol in this concentration should have the same value as that of potato tissues: -300 mOsm We also refer to the class data average and get such concentration by , and the difference from our data is about 3.2%. The difference could result from water evaporation since we are measuring weight to 4 decimal places, and this little difference is negligible. No controls were introduced in this experiment.

*Part E: Turgor Movement.*

From Text2, we observed that the leaflets would fold quickly in response to outside stimulations, and the result status is very similar to what Mimosa looks like under dehydrated conditions. When the outside environment stimulates Mimosa, the leaf cells will lose water quickly; thus, the cells are no longer turgid to support the leaflets' opening (Bidlack et al., 2020). However, when Mimosa is dehydrated, there is insufficient water for the plant cells to be turgid; thus, the leaflets will also fold.

In conclusion, carbon dioxide and water are critical materials for plants to photosynthesize. Plants must maintain equilibrium to get enough carbon dioxide without losing too much water by transpiration. Besides, water is also essential for turgor movement, stomata control, maintaining cell shape, and transfer of some substances like ions. Plant cells use membranes to protect cell organelles and other materials and allow selective material exchange into and out of cells, and control water flow by moving ions.

**References:**

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