**Exercise \_\_\_\_\_\_\_\_**

**Illuminating Photosynthesis:**

**Do different plants contain the same pigments?**

**\_\_\_\_\_\_\_\_\_\_**

**Purpose**

The purpose of this laboratory is to characterize the principle photosynthetic pigments for a better overall understanding of this complex biochemical process and determine if different plants contain the same photopigments.

**Lab Objectives:**

After completing this lab, students should be able to

1. Isolate and identify the light-harvesting pigments of Photosystems I and II using paper chromatography.
2. Determine the absorption spectrum of these pigments.
3. Compare the pigments present in different plants.

**Introduction**

**Importance of Photosynthesis**

Photosynthesis is arguably the single most important metabolic pathway, as it converts solar energy into the chemical energy used to reduce CO2 to glucose, the fuel that powers most life on Earth. Clearly, this complex pathway consists of two distinct stages.

During a two-step electron transport process, the “Light Reactions” consist of photon harvest by chlorophyll and carotenoid pigments and their subsequent conversion into ATP and NADPH. Known as the "Hill Reaction," the source of electrons for transport is the lysis of water. The remaining hydrogen ions will generate a gradient across the thylakoid membrane to permit the chemiosmotic production of ATP while the leftover oxygen proves a valuable byproduct for aerobic organisms.

The extremely endergonic reduction of CO2 is driven by the ATP and NADPH generated in the light reactions. Because this stage is only indirectly dependent on light, it is called the "Dark Reactions."

Although we will consider only the light reactions of photosynthesis in this experiment, remember that both light dependent and independent processes are essential for the solar-powered production of glucose.

**The Evolution of Plants**

What makes a plant a plant? You know one when you see one, don’t you? In this lab you will explore what of the prominent features most people use to identify a plant: the green color. Why are plants green? Are all plants green? What other pigments may be present in the plant cell? Are the same pigments present in all plants? To help us answer this question, you and your partner will be assigned to research one plant. Then in lab, we will tackle the “what pigments are present in plants?” and “are they the same in all plants?” questions.

**Pre-Lab**

You and your partner will choose one of the following plants to research:

* Ginkgo
* Larch
* magnolia
* swamp cypress
* dawn redwood
* taxus
* white pine
* arbor vitae
* Corn
* True Fern
* green algae

We want to learn about the evolutionary relationship between these plants. To do this, you and your partner will determine the following about your plant:

1. What is the genus and species name of your plant?
2. In which phylum is the plant classified? Class?
3. What are the major characteristics (structures) present in plants of that phylum?
4. What is the earliest evidence of your plant? In other words, how long has it existed on the planet? Include the Period and Era.
5. From previous research, what pigments are present in plant cells? What color does each appear? Where are they located within the plant cell?
6. Find and save a photo of your plant.

Summarize the information in a 1-2 page brief report. Do not write the question then answer it. Write the information as a flowing narrative.

Be certain to cite all sources so that you know exactly where you got the information that answered each question. This includes an **In-text citation** and a listing at the end of the paper **Works Cited**.

**Procedure**

**Part 1. Isolation and Identification of the Principle Photosynthetic Pigments.**

Paper chromatography is a quick, simple pigment separation method. The following methods was used to extract the pigments from each of the plants used in this lab. You will need the following materials from the front bench:

* A strip of chromatography paper 7.5 cm wide and trimmed to fit chamber.
* Chromatography chamber with lid and hook.
* ~25 ml chromatography solvent **(NOTE: Highly Flammable!).**
* 3 g deveined spinach, pinch of sand, 15 ml chilled acetone, and mortar and pestle.
* Pasteur pipette, 15 ml centrifuge tube, capillary tube, and paper towel.

Scissors, mm ruler, test tube rack with 5 (or more) capped tubes + 4 (or more) cuvettes.

* Extracted pigment for your assigned plant: ginkgo, larch, magnolia, swamp cypress, dawn redwood, taxus, white pine, arbor vitae, corn, true fern, or green algae.

1. Tear spinach into small pieces and place into a chilled mortar with 15 ml of ice cold acetone and a pinch of sand. Grind the leaves thoroughly for 1 minute with a chilled pestle.  **(NOTE: Acetone is also flammable.)**
2. Transfer the liquid and pulp to the capped centrifuge tube. Shake vigorously for 10 sec and place in the refrigerator for 10 min. During this time, the pulp will settle and additional pigment will be extracted from it.
3. With a pencil and a ruler, draw a line across the width of your chromatography paper about 3 cm from the bottom. The extract will be applied to this site (origin) and it must **not** be immersed in the solvent.
4. Using a capillary tube, streak at least 10 applications of the pigment extract along the line. The capillary tube is filled by immersing the tip in the extract. The flow from the capillary tube is controlled by finger pressure at the top. Allow each application to dry before making the next. Gentle blowing will facilitate the process. The final thickness of the streak should be no more than 6-7 mm.
5. Attach the streaked chromatography paper to the chamber lid and insert into the equilibrated chamber being careful to adjust the solvent volume so that it does not directly contact the streak. Allow the chromatogram to develop for 15-45 min. Stop the development before the solvent front reaches the top of the paper.
6. Remove the chromatogram from the chamber and hold by the top corner until dry. Using a pencil, mark the solvent front and the center of each band. Record the requested information in the data table 1 below.
7. Take a photo of your chromoatogram.

**Data Analysis:**

**Calculate the Rf values for each pigment.**

**R(f) values** are a measure of the rate of chromatographic component migration and thus indirectly a determination of component solubility within a given solvent. An R(F) value of 0.5 means that the component travelled 50% of the distance that the solvent moved.

Calculate the pigment R(F) values for your chromatogram by measuring the distance travelled (in mm) by each pigment and dividing it by the distance travelled by the solvent front.

Record your Rf  values in Data Table 1.

For a given solute and solvent at a specific temperature, this value is a constant.

**Data Table 1.** Chromatogram Data for \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Pigment** | **Color** | **Solute distance (mm) from origin** | **Distance of solvent front (mm)** | **Rf** |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |
|  |  |  |  |  |
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|  |  |  |  |  |

**Part 2.** **Absorption Spectra of the Principle Photosynthetic Pigments**

1. Obtain and label as many tubes as you have distinct bands on your chromoatogram.
2. Cut out each of the bands and place each in the appropriately labeled tube. To facilitate elution of the pigments, you may wish to cut each band into several very thin strips.
3. Add 4 ml of acetone to each tube and seal. **(Note: Remember that acetone is flammable.)**

* Allow the pigments to elute for 5 min, occasionally swirling the tubes.
* Invert the tubes several times to thoroughly mix the contents.

1. Using a pipette, transfer the pigment solutions to appropriately labeled cuvettes.
2. Using the Spectronic 20 and the indicated wavelengths, determine the absorption spectrum for each of your eluted pigments. The spec must be calibrated with the blank at each wavelength. Record this information in Data Table 2 or reproduce the table in an Excel spreadsheet.

**Data Table 2.** Absorbance Readings for Pigments.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Wavelength (nm)** |  |  |  |  |  |
| 400 |  |  |  |  |  |
| 410 |  |  |  |  |  |
| 420 |  |  |  |  |  |
| 430 |  |  |  |  |  |
| 440 |  |  |  |  |  |
| 450 |  |  |  |  |  |
| 460 |  |  |  |  |  |
| 470 |  |  |  |  |  |
| 480 |  |  |  |  |  |
| 490 |  |  |  |  |  |
| 500 |  |  |  |  |  |
| 510 |  |  |  |  |  |
| 520 |  |  |  |  |  |
| 530 |  |  |  |  |  |
| 540 |  |  |  |  |  |
| 550 |  |  |  |  |  |
| 560 |  |  |  |  |  |
| 570 |  |  |  |  |  |
| 580 |  |  |  |  |  |
| 590 |  |  |  |  |  |
| 600 |  |  |  |  |  |
| 610 |  |  |  |  |  |
| 620 |  |  |  |  |  |
| 630 |  |  |  |  |  |
| 640 |  |  |  |  |  |
| 650 |  |  |  |  |  |
| 660 |  |  |  |  |  |
| 670 |  |  |  |  |  |
| 680 |  |  |  |  |  |
| 690 |  |  |  |  |  |
| 700 |  |  |  |  |  |

**Data Analysis:**

1. **Plot the Absorption Data**

Using the data collected in data table 2, plot the absorbance spectra for all pigments on the same graph. You can set up an Excel spreadsheet and highlight the data range before you start recording absorbance data. The data points will be plotted as you record the data.

1. **Determine the absorption maxima for each pigment.**

For each pigment, determine the wavelength or wavelengths (there may be more than one) at which the pigment absorbs maximally. To which color in the visible spectrum does each wavelength correspond?

1. **Share data for your plant with the class.**

Upload your data to Google Sheets for the lab.

**Questions:**

1. What do the Rf values indicate about the relative solubilities of the pigments in the water and solvent phases?

2. Explain the relative solubilities of chlorophyll a and chlorophyll b in the water and solvent phases on the basis of molecular structure.

3. At what wavelengths is absorption a maximum for each of the various pigments? State the name of the pigment and the absorption maximum for each pigment.

4. How does the absorbance spectra of chlorophyll account for its color?

5. What advantage is there for a plant to have accessory pigments like xanthophylls and carotene that different absorption spectrum than that of chlorophyll?

1. Which pigments did all plants tested have in common?
2. Which plants contained pigments that were either unique to that plant or shared with a few other plants? What were the unique pigments?
3. What do you know about these plants that might account for the differences seen in these plants?
4. What are two questions that you now have about the evolution of plant pigments and photosynthesis that arose due to your experimental results and/or analysis of your data?

