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Chapter 35

Quantification of leaf chlorophyll content: Spectrophotometric estimation and physiological implications

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1. Introduction:

Chlorophyll, a green pigment found in plants and algae, is crucial for photosynthesis, converting light energy into chemical energy (Arnon, 1949). Quantifying chlorophyll content is essential for research in plant physiology, ecology, agriculture, and environmental monitoring. Spectrophotometric techniques are widely used due to their accuracy, sensitivity, and versatility (Harborne, 1998). They involve extracting chlorophyll pigments from leaf samples using organic solvents, determining their absorbance values at specific wavelengths. These methods offer high sensitivity, rapid analysis, and the ability to process large samples simultaneously. They are non-destructive, allowing repeated measurements on the same sample over time (Lichtenthaler, 1987; Tripathi et al., 2013a.b).

2. Materials required:

1. Fresh leaf samples
2. Acetone (99.5% pure)
3. 80% acetone solution
4. Distilled water
5. Mortar and pestle
6. Spectrophotometer
7. Test tubes or cuvettes
8. Aluminum foil or dark bags
9. Graduated cylinders
10. Absorbance tubes
11. Marker pen
12. Forceps
13. Vortex mixer

3. Procedure:

1. Harvest healthy leaves from the desired plant species.
2. Remove any debris or foreign particles from the leaves.
3. Quickly weigh the fresh leaf samples and record the weights.
4. Cut the leaves into small pieces using scissors.
5. Grind the leaf samples thoroughly in a mortar and pestle with 80% acetone solution until homogenous green slurry is obtained.
6. Transfer the slurry into labeled test tubes or centrifuge tubes.
7. Add sufficient 80% acetone solution to the tubes containing leaf slurry to make up a known volume (usually 5-10 mL).
8. Cap the tubes and vortex mix thoroughly for 1-2 minutes.
9. Incubate the tubes in a dark environment for 24-48 hours at room temperature to allow complete extraction of chlorophyll pigments.
10. Shake the tubes gently at regular intervals during the incubation period to ensure efficient extraction.
11. After the incubation period, centrifuge the tubes at low speed for 5 minutes to settle the debris.
12. Carefully transfer the supernatant containing chlorophyll extract to clean, labeled absorbance tubes.
13. Measure the absorbance of the chlorophyll extract at wavelengths of 664 nm (for chlorophyll a) and 647 nm (for chlorophyll b) using a spectrophotometer.
14. Use distilled water as a blank reference and set the spectrophotometer accordingly. e. Record the absorbance values for each wavelength.
15. Calculation of Chlorophyll Content:

    Calculate the concentration of chlorophyll a and chlorophyll b using the following equations:

    a. Chlorophyll a (mg/mL) = (12.7 × A664) - (2.69 × A647)
    b. Chlorophyll b (mg/mL) = (22.9 × A647) - (4.68 × A664)
    c. Total Chlorophyll (mg/g) = (Chlorophyll a concentration + Chlorophyll b concentration) × Dilution factor/ Leaf sample weight (g)

4. Precautions:

1. Handle leaf samples gently to avoid mechanical damage or bruising.
2. Use sharp scissors or blades to collect leaf samples to minimize stress and damage to plant tissues.
3. Ensure leaf samples are immediately placed in ice-cold acetone or ethanol to prevent chlorophyll degradation.

4. Work in dim or low-light conditions to minimize light exposure which can degrade chlorophyll.
5. Use glassware and equipment that are clean and free from contaminants to prevent interference with chlorophyll extraction.
6. Grind leaf samples thoroughly and uniformly to ensure efficient extraction of chlorophyll pigments.
7. Centrifuge or filter extracts to remove debris and insoluble material before measuring absorbance.
8. Measure absorbance of chlorophyll extracts promptly after preparation to prevent degradation.
9. Use appropriate solvents and wavelengths for spectrophotometric analysis according to the method being followed (e.g., acetone and wavelengths around 664 nm and 647 nm for chlorophyll a and b).
10. Prepare standard solutions of known chlorophyll concentrations to generate a calibration curve.
11. Perform measurements in triplicate or more to ensure accuracy and reproducibility of results.

5. References:

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