Meeting 1: Introduction to Laboratory Practical

Practical Class Content: Introduction to Laboratory Practical & Safety
Practical approaches
Guidelines to writing a formal lab report

Objectives:
1. To enable student to gain knowledge in laboratory exercise relating the fundamentals in plant physiology
2. To expose student to recent techniques in measurements and approaches in plant physiology
3. To enable students to plan, conduct, collect data and summarize data related to plant physiology research and write a report
4. To familiarize students with scientific presentation based on their research project

Laboratory Safety

For maximum safety and efficiency in the laboratory the following "rules" must be followed at ALL times.

General Rules:

1. You are responsible for your own safety and that of your neighbors. Work slowly and carefully and don't take chances. Don't fool around - that's when accidents happen.
2. No eating, drinking, smoking, chewing gum or tobacco, or applying cosmetics in the laboratory.
3. Shoes must be worn at all times. No sandals or open-toed shoes.
4. Do not work alone in the laboratory. You must always be supervised.
5. Report all accidents, no matter how small, to your lab assistant immediately.
7. Perform only authorized experiments.
8. Clean up all spills immediately. Report serious spills (i.e. acids, bases, organic solvents to your lab assistant.)
9. Always wash your hands before leaving lab. It is a good idea to wash your hands at intervals during your experiments.
10. Learn the location of appropriate safety equipment including first-aid kit, fire extinguisher, safety shower, eyewash station, and acid/alkali spill clean-up materials.
11. Many living organisms are biological hazards (i.e. poisonous, pathogens) and must be handled with extreme care. Contact your lab assistant for proper handling techniques.

Lab Etiquette:

1. You are responsible for the care and proper use of all assigned equipment.
2. Never pipette reagents directly from stock bottles. Always transfer required amount to a beaker and pipette from it. Or, use the pipette provided with the reagent.
3. Do not return unused reagents to the stock bottle.
4. Label all materials with your name, date, and section. Do not write directly on glassware, but write on tape. Use a pencil or special lab marking pen.
5. Never heat flammable solvents (i.e. methanol) over an open flame. Use a steam bath, heating mantle, etc.
6. Do not use broken or chipped glassware. Return it to your lab assistant.
7. Be certain to read the labels on reagent bottles before using them. Use the proper reagents in your experiment.
8. Do not bring materials to be used by the entire class to your table.
9. The laboratory door must remain closed for efficient ventilation.
10. Only materials required for your experiments (i.e. notebook, pen, equipment) should be on your lab table. Leave books, coats, etc. in the hall.
11. Clean up your work space and materials at the end of your experiment. Wash your dishes and remove all labels.
12. Dispose all materials properly. Consult your lab assistant for proper techniques. Do not throw solids in the sink.
13. Do not open Petri dishes contaminated with fungi or bacteria in the laboratory. They must be autoclaved first or thrown away. Consult your lab assistant.
14. Before using any equipment, be sure you know how to operate it. You will be charged for breakage due to improper or careless handling. This also applies to any equipment rendered useless because of failure to clean it properly.
PRACTICAL MANUAL AGR3301-PJJ

GOGGLES/GLOVES:

Safety goggles and/or gloves will be required for several experiments. You must wear the appropriate safety equipment to participate in these labs. I will have some available for you - or, you can bring your own (preferable).

Practical approaches

1. The student will be divided into 8 – 10 groups with not more than 8 members in a group.
2. For each practical session, student will be assigned to conduct experiments and to monitor, collect data and to produce scientific reports from each laboratory session.
3. Part of the laboratory work will be based on a continuous short experiment and the students are responsible for their experiments outside the scheduled laboratory hour to collect data for report writing.

Formal Lab Reports

Laboratory reports should be written in English with the format and style of articles. Familiarize yourself with a recent edition of lab reports writing for the details of organization, headings, methods of citing references, methods of presenting data, etc. The following briefly summarizes this information. Finally, although the style of writing of a lab report will differ from that of an English essay, lab reports should be well-written and grammatically correct. The report submission and evaluation will be on individual basis. Each student has to write her/his own report and the grading will be based on the depth of the reports.

The major sections of a laboratory report are:

I. Title
II. Introduction
III. Materials and Methods
IV. Results
V. Discussion
VI. Literature Cited
I. Title: - Keep the title of your paper as brief as possible. Capitalize the first letter of each major word. The title should be descriptive and give the reader an immediate indication of the subject of your paper.

II. Abstract: An abstract is a brief summary of your paper.

III. Introduction: This section discusses your reasons (i.e., question) for conducting the experiment, the purpose of your experiment, and pertinent background information concerning your experimental topic. Your hypothesis should be included in some form. This section should include a review of pertinent literature related to your project.

IV. Methods and Materials: This is a written description of the experimental procedures. Labeled diagrams of complicated apparatus may supplement the written description. This section should be explicit enough so that another investigator could repeat your experiment. One of the criteria of science is that it is repeatable; that is, others would get the same results if they followed your methods. Be as specific as possible indicating how much, how long, how many types, where samples were collected and when, types of equipment used, etc. This section should be written in past tense. If the procedures you used are published elsewhere, including the lab manual, simply cite that reference; however, you must still give a brief summary of each major activity. Be sure to include the source, identity and method of preparation of your plant materials.

V. Results: This section is a written description of the results of your experiment. It includes all the information gathered during the course of the experiment, including graphs, tables and figures. It is recommended that you graph data whenever possible - it is much easier to interpret data when they are presented in a graph form than when they are in tabular form. Again, use past tense. Please append a copy of your raw data to the report and provide a sample calculation(s). You may photocopy shared group data. However, unless otherwise stated you must prepare all final graphs and tables on your own.

VI. Discussion: This section should: (1) offer an explanation for the results; (2) interpret the results; (3) indicate if the results were expected and why or why not? (4) discuss any previous studies or information related to the experiment and compare the results of the two experiments; and (5) suggest the significance of the results and experiment. If the experiment didn't work out, don't just say "experimental error," suggest reasons for the failure.
VII. Literature Cited: This is the last section of a scientific paper. This section lists, alphabetically by author and numbered consecutively, the references cited in the body of the paper. The abbreviations of journals should conform with BIOSIS - List of Serials. Many different formats for citing references are used in the scientific literature. As expected, the form we shall follow is that used in Plant Physiology and is summarized below:

**Book**


**Book Articles**


**Journal Article**


**No Author or Editors**

Title of Booklet, Pamphlet, etc. (1975) Publisher (or Company), City
Online

Author A (year of publication) Title. access date. web address

http://www.employees.csbsju.edu/ssaupe/biol327/Lab/Lab-Reports.htm

Citing References: References are cited in the text in two ways:

1. Singer (1956) reported that *Pluteus salicinus* is common in some areas of North America and Europe; or

Presentation (Tentative)

Each group will be present results to other class members toward the end of the semester. Each member must participate during presentation. Format and contents of presentation shall follow your final lab reports. Each group will be allocated 20 minutes for each title. Any changes regarding presentation will be informed to students.
Meeting 1:
  a. Growth analysis

**Practical Class Content:**  Introduction to Growth Analysis and Crop Dry Matter Accumulation (Measurement of plant productivity)

**Learning objectives:**

1. To learn what are the most relevant methodologies to measure the daily performance of crop canopy
2. Why does plant-to-plant variability make the measurement of plant productivity so difficult?
3. To know what is radiation use efficiency (RUE)
Growth analysis: Experiments with Corn to Demonstrate Plant Growth & Development

Objectives
1. To observe changes of dry weight during germination and exchange to the log, as it grows
2. To change the values given to the graph form. This method usually been used in scientific writing report where the experimental result can easily determined.

Methods
The experiment with corn to demonstrate plant growth and development has been done. This small experiment was well maintained followed the farm management practices. Data were collected at 0, 3, 5, 7,… 17 days after sowing for determination of dry weight. The roots were carefully washed free of adherent soil/sand particles. The shoot, root and seed samples were then placed in the respective labeled paper-bag and dried in an oven at 98°C for 2 days to constant weight.

Results
The result of dry weight of each part is shown in Table 1:

<table>
<thead>
<tr>
<th>Day after germination</th>
<th>Shoot dry weight (a)</th>
<th>Root dry weight (b)</th>
<th>Seed dry weight (c)</th>
<th>Total dry weight (a) + (b) + (c) = (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mg/plant loge</td>
<td>mg/plant loge</td>
<td>mg/plant loge</td>
<td>mg/plant loge</td>
</tr>
<tr>
<td>0</td>
<td>0.0</td>
<td>0.0</td>
<td>-</td>
<td>320.0</td>
</tr>
<tr>
<td>3</td>
<td>11.5</td>
<td>2.4423</td>
<td>5.1</td>
<td>213.8</td>
</tr>
<tr>
<td>5</td>
<td>19.8</td>
<td>2.9857</td>
<td>9.3</td>
<td>200.0</td>
</tr>
<tr>
<td>7</td>
<td>52.2</td>
<td>3.9551</td>
<td>58.5</td>
<td>178.8</td>
</tr>
<tr>
<td>9</td>
<td>100.1</td>
<td>4.6062</td>
<td>146.4</td>
<td>124.3</td>
</tr>
<tr>
<td>11</td>
<td>165.7</td>
<td>5.1103</td>
<td>241.1</td>
<td>89.2</td>
</tr>
<tr>
<td>15</td>
<td>420</td>
<td>6.0402</td>
<td>501.8</td>
<td>1010.8</td>
</tr>
<tr>
<td>17</td>
<td>600</td>
<td>6.3943</td>
<td>753.0</td>
<td>1441.0</td>
</tr>
</tbody>
</table>
Discussions

From the table above:

a. Plant growth by dry weight

i. How shoot (a), root (b), seed (c) and total (d) dry weight can change with time? By using information above, you can draw a graph to see the trend of each part of measurement.

ii. From the graph, observe the change in weight of each part by time. Get the relationship between seed dry weight and total dry weight in the beginning of growth. How long for total dry weight decrease and when its increase again? Why this happened?

From the graph, calculate the slope for total dry weight and fill up the Table 2 below.

<table>
<thead>
<tr>
<th>Duration</th>
<th>Begin</th>
<th>End</th>
<th>Slope</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 7 to 9</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Day 9 to 11</td>
<td></td>
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<tr>
<td>Day 11 to 12</td>
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<tr>
<td>Day 13 to 15</td>
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</tbody>
</table>

How the slope can change with time? Condition where the slope increase with time is called exponential form.

b. Plant growth by dry weight change to log<sub>e</sub>

Exchange the numbers to log<sub>e</sub> (natural logarithm):

Method: \[ \text{If } X = X \times 10^p \rightarrow \log_e X = \log_e x + \log 10^p \]

Example:

\[
\begin{align*}
\log_e 2.411 &= 0.8800 \text{ (from book)} \\
\log_e 24.11 &= \log_e 2.411 + \log_e 10^1 \\
&= 0.8800 + (2.3026 \times 1) \\
&= 3.1826
\end{align*}
\]
log$_e$ 241.1 = log$_e$ 2.411 + log$_e$ 10$^2$
= 0.8800 + (2.3026 x 2)
= 5.4852

i. Determine the value of W, X, Y and Z from the table given. Show the calculation.

ii. Draw a graph for log$_e$ total dry weight with time. From your observation, does the slope change with time between day 5 and day 17?

Calculate slope average from day 5 to day 17 (from the graph):
log$_e$ dry weight ($\log_e W_1$) at 5$^{th}$ day ($t_1$) =
log$_e$ dry weight ($\log_e W_2$) at 17$^{th}$ day ($t_2$) =
Slope = \frac{\log_e W_2 - \log_e W_1}{t_2 - t_1}

This slope also known as relative growth rate (RGR) for that plant. Unit: mg/mg/day. This shows the efficiency of that plant to increase the dry weight over a time interval in relation to the initial weight.
Meeting 1: 
  Determination of Leaf Area I

Introduction

Leaves are a plant's main photosynthetic organs. Leaf structure is closely associated with its photosynthetic function. Through the process called photosynthesis plants absorb light through their leaves and use it to split water molecules into hydrogen and oxygen molecules. The oxygen is released into the atmosphere and the hydrogen is combined with carbon dioxide from the atmosphere to create sugar to feed the plant. It is clear that the plant's ability to create food is dependent on the surface area of its leaves. That is why photosynthesis rate is directly related to the leaf surface area. To arrive at the rate of photosynthesis, therefore, you must calculate the leaf surface area of each plant, because most stomata are found in the lower epidermis, you will determine that surface area. There are a few methods to measure leaf surface area, such as:

  a) Leaf surface area by weight
     By tracing the leaf area onto a piece of paper and cut off the area traced. Weight the cut area. The weight can be related with the paper weight which the area is known.

  b) Count the number of squares on graph paper
     To trace the leaf area on a piece of 1 cm grid of graph paper and count the total square cm on the graph.

  c) Using Automatic Leaf Area Meter (ALAM)
     By using a device called Automatic Leaf Area Meter (ALAM) that can be used to measure leaf surface area automatically. This method is easier and faster.

Objectives

1. To determine the leaf surface area by using these three methods above
2. To relate method of leaf surface area determination with another. Relationship is determined by plotting graph and calculates the relationship statistically.
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Practical Class Materials: Compound leaves
Graph paper
Scissor
Automatic Leaf Area Meter (ALAM)

Procedures

a) Determination of leaf surface area by weight

Each group will be given 10 compound leaves. Determination of leaf surface area as follow:

a. Measure the length and width of 10 graph papers and weighted each of the graph paper. Fill the results in Table 1.
b. Numbered the leaves from one to ten and mark the leaflet. Trace the leaves on the graph paper and number the traced paper. Cut off the traced papers and weigh each one of them. From the previous knowledge on average area per gram of paper, calculate the area of each leaf and fill the result in Table 2.

b) Determination of leaf surface area by count the number of squares on graph paper

Count the number of squares centimeters which are completely covered from exercise 1b. above. Fill up the results in Table III. Estimate the area of the partial squares. Here is a simple method for this estimate. Count a partial square if it is at least half covered by the leaf, do not count partial squares that are less than half covered. Do not include the area of the stem (petiole) in your calculations.

c) Determination of leaf surface area by using ALAM

Determine the area of each traced leaf paper and fill up the results in Table IV.

d) Relationship between leaf surface areas

From the result in Table I to IV, draw a scatter diagram on graph paper for:
1. Relationship between leaf area method a (y axis) with leaf area method c (x axis), and
2. Relationship between leaf area method b (y axis) with leaf area method c (x axis).
Results

Table I: Weight and area of graph paper

<table>
<thead>
<tr>
<th>Paper</th>
<th>Weight, W (g)</th>
<th>Length, P (cm)</th>
<th>Width, L (cm)</th>
<th>Luas, P\times L (cm²)</th>
<th>Luas/W (cm²/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paper 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Paper 2</td>
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</tr>
</tbody>
</table>

Table II: Leaf surface area by weight

<table>
<thead>
<tr>
<th>Leaf No.</th>
<th>Weight (g)</th>
<th>Area by calculate (cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
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<td>2</td>
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<td>10</td>
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</table>
Table III: Leaf surface area by count the number of squares on graph paper

<table>
<thead>
<tr>
<th>Leaf No.</th>
<th>Leaf area (cm²)</th>
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</thead>
<tbody>
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<td>1</td>
<td></td>
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<td>2</td>
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<tr>
<td>10</td>
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</tbody>
</table>

Table IV: Leaf surface area by ALAM

<table>
<thead>
<tr>
<th>Leaf No.</th>
<th>Leaf area (cm²)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
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<tr>
<td>2</td>
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<td>..</td>
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<td>9</td>
<td></td>
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<tr>
<td>10</td>
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</tbody>
</table>

Discussions

From the graph of d1 and d2, estimate the linear relation graph between values from y and x axis only.

- a. What is your opinion about relationship between those methods mentioned above?
- b. What is your opinion about the estimation of linear relationship graph drawn?
Meeting 1:  
c. Determination of Leaf Area II

Practical Class Content:  Traced paper leaves (from DLAI)  
                     Calculator

Introduction

In previous practical exercises, relationship between method a and b was determined roughly. The accurate method is by using linear regression between factor a and b. Linear regression attempts to model the relationship between two variables by fitting a linear equation to observed data. One variable is considered to be an explanatory variable, and the other is considered to be a dependent variable. For example, a modeler might want to relate the weights of individuals to their heights using a linear regression model. Before attempting to fit a linear model to observed data, a modeler should first determine whether or not there is a relationship between the variables of interest. This does not necessarily imply that one variable causes the other (for example, higher SAT scores do not cause higher college grades), but that there is some significant association between the two variables. A scatterplot can be a helpful tool in determining the strength of the relationship between two variables. If there appears to be no association between the proposed explanatory and dependent variables (i.e., the scatterplot does not indicate any increasing or decreasing trends), then fitting a linear regression model to the data probably will not provide a useful model. A valuable numerical measure of association between two variables is the correlation coefficient, which is a value between -1 and 1 indicating the strength of the association of the observed data for the two variables.

A linear regression line has an equation of the form \( Y = a + bX \), where \( X \) is the explanatory variable and \( Y \) is the dependent variable. The slope of the line is \( b \), and \( a \) is the intercept (the value of \( y \) when \( x = 0 \)).

Procedures

In this practical class, you will determine relationship between lengths of centre leaf (\( X \)) with total leaf area (\( Y \)) so that total leaf area can be determined with just measure the length of centre leaf only. This method is suitable for plant with big leaves like palm oil. From 10 traced paper leaves (previous practical), measure the length of centre leaf and record in Table 1. Fill up the column for actual leaf area, taken from method c in practical Determination of Leaf Area I.
Table 1

<table>
<thead>
<tr>
<th>Leaf No.</th>
<th>Measurement of centre leaf (cm)</th>
<th>Actual leaf area , y (cm²)</th>
<th>y²</th>
<th>xy</th>
</tr>
</thead>
<tbody>
<tr>
<td>x</td>
<td>x²</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>1</td>
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</table>

\[ \sum x \quad \sum (x^2) \quad \sum y \quad \sum (y^2) \quad \sum xy \]

\[
\frac{\sum x}{(\sum x)^2} \quad \frac{\sum (x^2)}{(\sum y)^2} \quad \frac{\sum y}{(\sum y^2)} \quad \frac{\sum xy}{(\sum x)^2} \]

iii. Fill up Table 1
iv. Draw a scatterplot on graph paper for relationship between factor x and y. Roughly, draw linear line that relate x and y.
v. Get the conclusion for relationship between factor x and y using linear regression, \( y = a + bx \). Draw a graph by using this equation on the same graph paper from b. Compare these two lines.
vii. Write your opinion about suitability of leaf area determination by measure length of centre leaf based on result in d.
viii. Gather all conclusions and correlation coefficient findings in your class.

**Method to measure linear regression and correlation coefficient**

a. Linear regression

Relationship between factor x and y:

\[ y = a + b (x - x) \rightarrow \text{where } y = \text{value (estimate for y)} \]

\[ a = \text{constant} \]
\[ b = \text{regression coefficient} @ \text{slope for line regression} \]
Value for a and b can be determined as follows:

\[
a = y - bx
\]

\[
b = \frac{\sum(x - x)(y - y)}{\sum(X - X^2)}
\]

\[
= \frac{\sum(xy) - \sum(x)y}{\sum(x^2) - [\sum(x)]^2/n}
\]

After value b determined, y is:

\[
y = a + b(x - x)
\]

\[
= a - bx + bx
\]

By inserting x value, y value can be determined. Two y values can be identified with inserting two x values and that two values can be as value point on the graph.

b. Corrélation coefficient (r)

\[
r = \frac{\sum(x - x)(y - y)}{\sqrt{[\sum(x - x^2)] [\sum(y - y^2)]}}
\]

\[
r = \frac{\sum(xy) - [\sum(x)]y}{\sqrt{[\sum(x^2) - (\sum x)(x)] [\sum(y^2) - (\sum y)(y)]}}
\]

To identify whether \( r \) shows a significant corrélation relationship (statistically) or not is by checking correlation coefficient table. For 10 pairs, \( r \) value with \( df = 10 - 2 = 8 \), at \( p \leq 0.05 \),

\[
r_{df8} = \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots \ldots
Meeting 2:
a. Seed Viability

Introduction

The food for the germinating seedling may be stored in part of the embryo itself, such as the fleshy cotyledons of a bean seed, or it may take other forms including endosperm, which is a special starch-rich storage tissue that surrounds the embryo. A seed is officially considered to have germinated when the young root, called the radicle, emerges from the seed coat. To germinate, a seed requires three things – water, oxygen, and a suitable temperature. Water uptake, also called imbibition, is the first stage of seed germination. During this process the dry seed, which typically has water content of less than 10%, absorbs water and swells. This process serves to hydrate the dry components of the seed and active the metabolic machinery necessary for germination. Among the early metabolic activities occurring in the seed is the breakdown of starches stored in the seed into simple sugars that can be used for energy and building blocks for necessary cellular structures. A seed that has not germinated because it is lacking one or more of the necessary requirements for germination is termed quiescent. These seeds are simply "resting", waiting for the appropriate conditions for germination. Given water, oxygen and/or a suitable temperature, a quiescent seed will germinate. However, even if given the proper conditions, a seed may not germinate. These seeds may fail to germinate because the seed is either dormant or "dead". Dormant seeds have the potential to germinate but are prevented from doing so by some mechanism. Thus, even though all the proper growth conditions are present, they don't germinate unless they have been "primed" and there dormancy mechanism has been overcome. There are many dormancy mechanisms in seeds.

The purpose of laboratory testing of seed germination is to assess seed quality or viability and to predict performance of the seed and seedling in the field. Several different kinds of testing are available depending on the type of seed to be tested, the conditions of the test, and the potential uses of the seed.

Germination percentage is a good measurement of seed viability. One disadvantage of conventional germination tests is that they require a waiting period of two weeks or more. The tetrazolium test (TZ- test) is a quick chemical test that can be conducted in a short period of time with minimal equipment. In this test, a seed is incubated in a dilute (0.1%) solution of 2,3,5-triphenyltetrazolium chloride.
Initially, the TZ solution is colorless but changes to read (Triphenyl formaza) when it comes into contact with hydrogen (reduction) derived from enzymes in the respiration process. Embryos showing active respiration are considered ‘viable’ and turn red.

Objectives

1. To determine the seed viability of maize and bean seeds using TZ test.
2. To compare TZ test with conventional germination test.

Exercise 1: Tetrazolium test (Chemical test which gives a quick estimate of seed viability (24-48 hrs.)

Maize

1. Randomly select 10 seeds for 2 replications. The seeds must be hydrated by soaking in water for 12 hours or overnight.
2. Cut the seeds longitudinal through the midsection of the embryo and through part of the endosperm. Keep only one half for the staining, discard the other.
3. Place the seeds into petri dish and fill tetrazolium chloride solution enough to cover the seeds and allow for absorption.
4. Covers petri dish and incubate in the oven (35°C) for approximately 1 hour or until seed changes to red (varies according to seed conditions, species, temperature, solution concentration).
5. Remove seeds out from the tetrazolium solution, rinse 2-3 times in water and then evaluate according to the staining pattern (Diagram is provided).
6. Identify staining areas to determine the viability. Parts include scutellum, coleorhizae, radical and plumule.

Bean

Similar procedure as described (a) except the seed coat need to be removed before splitting the bean into half.

When working with tetrazolium, use gloves and forceps to handle the seeds.
Exercise 2: Germination test (test determines the percentage of normal seedlings that develop under ideal conditions. The percentage of hard or dormant seed is also determined and reported)

a) Sand media (maize and bean)

1. Randomly select 10 seeds for 2 replications for each crop.
2. Fill clean and sterile sand into trays.
3. Moisten the sand to the field capacity. Do not use excess water.
4. Sow seeds to the depth of 2-3x of the seed diameter in rows.
5. Check regularly the moisture level and sprinkle water when necessary.
6. Label the species, date of sowing and replicate.
7. Count the germinated seeds after 7 days for maize and 9 days for bean

b) Paper towel media (maize and bean)

1. Randomly choose 10 seeds for 2 replications for each crop
2. Moisten two sets of paper towel. The goal is to reach its saturation point.
3. Place seeds in rows on one set of moist paper towel and cover over the seeds with the other set.
4. Roll the paper loosely and place the rolled paper towel in the tray
5. Cover the tray with cling film to prevent the towels from drying out.
6. Place the tray in an upright position in a warm and not direct sunlight
7. Keep the paper towel evenly moist
8. After 5 to 7 days, unroll the paper carefully to avoid tearing the paper or damaging the roots of the young seedlings. Count the number of germinated seed with intact tap roots and shoots.
9. Do not count moldy seeds or diseased seedlings.

Germination percentage (GP) is an estimate of the viability of a population of seeds. The equation to calculate germination percentage is:

\[ \text{GP} = \frac{\text{total no. of seeds germinated}}{\text{total no. of seeds used}} \times 100 \]
Results

<table>
<thead>
<tr>
<th>GP</th>
<th>TZ-test</th>
<th>Sand media</th>
<th>Paper towel media</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Maize</td>
<td>Bean</td>
<td>Maize</td>
</tr>
<tr>
<td>Replication 1</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Replication 2</td>
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<td></td>
<td></td>
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<tr>
<td>Average</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Draw a graph to show any relationship between these 3 methods for both crops and discuss the result.

From the class result, compare between chemical and conventional treatment by answer the question below:

1. Why germination test is so important in agriculture sector.

2. Why maize seed considered not viable if the scutellum has not change to red color by tetrazolium even the plumule and radical had changed to red color?

3. Can TZ differentiate between dormant and non-dormant seed? Give the reason.
Meeting 2:
b. Seed Moisture Content

Introduction

The seed moisture content (mc) is the amount of water in the seed. It is usually expressed as a percentage on wet weight basis in any seed-testing laboratory. The seed moisture content is the most vital parameter, which influence the seed quality and storage life of the seed. Seed moisture content is closely associated with several aspects of physiological seed quality. For example, it is related to seed maturity, optimum harvest time, mechanical damage, economics of artificial seed drying, seed longevity and insect & pathogen infestation.

The ease or difficulty in moisture management after harvest depends to a great deal on the climatic conditions during seed maturation and harvest. If the natural field environment does the job of drying the seed most storage problems are minimized. If not, drying has to be done artificially, and in some cases this approach can be complex and expensive. In either case, the most important, urgent and crucial requirement when the seed is being harvested is to measure the moisture content to see it is at a safe level. This simply highlights the importance of moisture. In some crops the urgency is higher than in others and the higher the moisture the greater the urgency safe for storage of most seeds. If the natural environment does this job you have a masterful production plan. Seeds stored at higher level exhibit increased respiration, which leads to heating and fungal invasion, which leads to poor seed viability and vigor. The higher the moisture content the worse the problem would be if not dried soon. Low moisture content in the seed to be stored, on the other hand, is the best prevention for all moisture derived problems. The lower the moisture content (below 13%), the longer seeds can be stored provided that the moisture level can be controlled throughout the length of storage. It has been reported that seed moisture content of about 6% is optimum for storage of most crop species for maximum longevity. Seed moisture content fluctuates with the changes in relative humidity. However, drying temperature must not eliminate other solutes (other than water) from the seeds.

Objective

a. To evaluate seed moisture content.
b. To determine the drying temperature for various seed types.
Technique: Constant temperature oven drying method

a) Low constant temperature (105°C for 16 hours)

This method has been recommended for seed of the species rich in oil content or volatile substances.
eg. *Allium* sp., *Glycine max*, *Arachis hypogea*, *Capsicum* spp seeds etc.

b) High constant temperature (130°C for one hour)

This method can determine moisture content faster and suitable for almost species except seed containing high volatile oil. The sample is dried to a period of four hours for *Zea Mays*, two hours for other cereals and one hour for other species Seeds from *Phaseolus* spp, chrysanthemum, *Cucurbita* spp, *Daucus carota* and etc are suitable for this method.

Methods

1. Weigh an empty petri dish with its cover.
2. Place approximately 4-5g of seeds in petri dish for each replication (maize and bean with 2 replications). Use analytical balance with 3 decimal places.
3. Place petri dish containing seeds at desired temperature in a preheated oven.
4. After 1 or 16hr (according to temperature), take the petri dish out of the oven and allow them to cool in desiccators (preferably).
5. Weigh the sample and calculate the moisture content.

Results

Compute the moisture content (MC) for each sample using the equation:

\[
MC = \frac{\text{Initial Weight}[A] - \text{Final Weight}[B]}{\text{Initial Weight}[A]} \times 100\%
\]
Record all data in the table given:

<table>
<thead>
<tr>
<th>Grain</th>
<th>T (°C)</th>
<th>Rep</th>
<th>Fresh wt. (g)</th>
<th>Dry wt. (g)</th>
<th>% Moisture content</th>
<th>Ave of % moisture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize</td>
<td>130</td>
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<td>II</td>
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<tr>
<td>Bean</td>
<td>130</td>
<td>I</td>
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<td>105</td>
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<td>II</td>
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</tbody>
</table>

Discuss the differences or similarities between both methods. Write approximately 100 words about ‘Seed moisture content and its important in agriculture’.
Meeting 2:
c. GA and Growth

Introduction

Gibberellic Acid-3 (GA-3) is a naturally occurring plant growth regulator which may cause a variety of effects including the stimulation of seed germination in some cases. GA-3 occurs naturally in the seeds of many species and is produced commercially by growing *Gibberella fujikuroi* fungus cultures in vats, then extracting and purifying the GA-3. Presoaking seeds in GA-3 solution will in many cases cause the rapid germination of many types of highly dormant seeds which would otherwise need cold treatment, after-ripening or ageing, or other prolonged pretreatments. Many different types of dormancy are overcome with GA-3 treatment. Not all seeds respond well. A great deal of research needs to be done to determine which species benefit, and the proper concentration of GA-3 for each type.

Objectives

1. To study the influence of exogenous GA application on bean seedling growth
2. To prepare stock solution and dilution

Materials and methods

Bean seeds and GA-3 solution with concentration of 800 ppm (dissolve 0.800 g of GA-3 in water, and make up to 1 L)

1. Using a dilution technique, prepare 20ml of GA-3 solution with the following concentrations: 400, 300, 200, 100 and 50 ppm
4. Soak 5 seeds in each solution concentration for an hour and then transfer into pot containing a rooting media.
5. Record the number of seeds germinated daily to determine the germination speed and % of germination.
6. After germination, measure the seedling height or length of internodes at two days interval for a period of two weeks.
Mean germination time (MGT) is calculated by following equation:

\[
\text{Mean Germination Time (MGT)} = \frac{\sum Si \cdot Di}{\sum N}
\]

- \(Di\): Day during germination period (between 0 and 24 day).
- \(Si\): Number of germinated seeds per day
- \(N\): Sum of germinated seeds

**Results**

Record daily data for seed germination and at 2 days interval for seedling height for period of 2 weeks:

<table>
<thead>
<tr>
<th>Day after treatment</th>
<th>400 ppm</th>
<th>300 ppm</th>
<th>200 ppm</th>
<th>100 ppm</th>
<th>50 ppm</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
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<tr>
<td>1</td>
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<td>5</td>
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<td>6</td>
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<td>7</td>
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</tbody>
</table>

Prepare for a class registration to compare the different between five GA concentrations. Based on class results, draw a graph and discuss seed germination and seedling growth in respond to GA treatments.
Meeting 2:
d. Auxin and Root Initiation

Introduction

Auxin, also known as indole-3 acetic acid (IAA), is a well-studied plant hormone that is responsible for plant growth in response to biotic and abiotic stresses. Usually, synthetic auxins like α-Naphthaleneacetic acid (αNAA) and 2,4-Dichlorophenoxyacetic acid (2,4-D) are used as herbicides. They have been in use as herbicides for the past fifty years due to their high effectiveness and cheap cost. Adequate production of the plant hormone auxin or indole-3-acetic acid (IAA) can efficiently promote plant root growth. Although auxin is a growth promoting hormone, it can be a metabolic burden to plants at high concentrations and therefore toxic. Synthetic auxins are extremely stable and can persist in the soil for weeks, which is why they are extremely effective herbicides. IAA, on the other hand, is chemically labile and can be easily metabolised by the plants.

The uses of synthetic auxins in horticulture can be traced directly to the natural roles of IAA in the plant. In general, compounds such as α-naphthalene acetic acid (NAA) are used because they resemble IAA in action but are resistant to degradation by plant enzymes. Auxins are used for a variety of agricultural purposes, including: promotion of rooting of cuttings. The base of the cutting is dipped in a powder containing NAA or indolebutyric acid (IBA) prior to planting. Root initiation tests are best known for their role in evaluating materials and cultural techniques for plant propagation purposes. In the laboratory, these tests have been used in studies of the growth and elaboration of plant parts, and have aided the characterization and identification of naturally occurring growth-substances.

Objectives:

1. To study the effects of various auxin concentrations on rooting and root growth
2. To preparation of stock solution and dilution
Materials and methods

You will be provided with Coleus sp. cuttings and indole-3-butyric acid (IBA):

1. Prepare 100 mgL⁻¹ stock solution
2. Using the stock solution, prepare 50 ml solution with the following concentrations: 75, 50, 25 and 0 mgL⁻¹ IBA. Use a dilution technique
3. Immerse 5 cuttings in each solution for 60 minutes
4. Firm the cuttings into rooting media and label according to treatment. Mist water daily to avoid cuttings from dehydrates.
5. All cuttings will be evaluated after 2 weeks. Count the number of rooted cuttings. Cut the roots and record for root length and root fresh weight. The root samples are then place in the oven at 70°C for 48 hours, for root dry weight.

Results

Record all data in the table given:

<table>
<thead>
<tr>
<th>IBA conc. (mgL⁻¹)</th>
<th>Rep</th>
<th>Root length (cm)</th>
<th>Root fresh wt. (g)</th>
<th>Root dry wt. (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>75</td>
<td>I</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>II</td>
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<tr>
<td>50</td>
<td>I</td>
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<td></td>
<td>II</td>
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<tr>
<td>25</td>
<td>I</td>
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<td></td>
<td>II</td>
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<tr>
<td>0</td>
<td>I</td>
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<td></td>
<td>II</td>
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</tbody>
</table>

Prepare for a class registration to compare the different between four IBA concentrations. Based on class results, draw a graph and discuss rooting and root growth in respond to IBA.
Meeting 3:
 a. Determination of Plant Water Status

Introduction

Plant tissues contain large amounts of water, and even larger amounts must be supplied to replace the water lost in transpiration. Part of the problem is that plants vary in their response to water. A better approach is to measure the water status of the plant. The differences in water use between species are then included in the measurements, and the varying effects of rainfall and evaporation are integrated as well. Methods to measure plant water status are based on measures of plant water content that are informative when compared to other tissue properties. Typically, the water content is compared to the tissue dry weight or is expressed as a percentage of the maximum water the tissue can hold. Tissue water content can be determined by oven dry until the dry weight constant. This method is good to differentiate water content for tissue parts or other crop species.

\[
\text{Moisture content} = \frac{\text{Fresh wt} - \text{Dry wt}}{\text{Fresh wt}} \times 100\%
\]

Other method is to determine relative water content (RWC), where RWC is a useful indicator of the state of water balance of a plant essentially because it expresses the absolute amount of water, which the plant requires to reach artificial full saturation. Thus there are relationships between RWC and water potential (will be discussed in Week 9). This relation varies significantly according to nature and age of plant material. The RWC express the water content in percent at a given time as related to the water content at full turgor:

\[
\text{RWC} = \frac{\text{fresh weight} - \text{dry weight}}{\text{saturated weight} - \text{dry weight}} \times 100\%
\]

Water makes up most of the mass of plant cells. In each cell, cytoplasm makes up only 5 to 10% of the cell volume and the remainder is a large-filled vacuole. Thus RWC is a measure of the relative cellular volume that shows the changes in cellular volume that could be affecting interactions between macromolecules and organelles.
B)  Plant water status – Plant moisture content (MC, %)

Objective

1. To determine MC at different part of plant
2. To compare duration of drying time

Methods

iii. Obtain 2 replications of Ipomea batatas or Amaranthus sp. with appx. 200g per replication
iv. Separate into leaf, stem and root and weight (Fresh weight)
v. Put each part in paper envelope separately and dry them in the oven at 70°C for 15, 23, 38 and 46 hour.
vi. Immediately weight the sample once taken out from the oven (Dry weight)
vii. Calculate percentage of water content base on fresh weight and fill up the Table 1
viii. Plot % MC vs. time (hour). Draw the best fit line for your data.
ix. From the graph, determine the duration of drying time to constant weight for each part.

C)  Plant water status – Relative water content (RWC, %)

Objective

a. To determine RWC at different water availability (100% and 50% of MC)
b. To compare RWC between two plant species

Methods

1. Select the young and fully matured leave for each treatment (4 replications)
2. Punch 10 disks out of a leaf by using No. 8 cork borer per plant per replication per treatment. The punch must be sharp to minimize cut-edge effects
3. Immediately weight the leaf disks (fresh weight) and place it in a petri dish.
4. Soak the leaf disks in distilled water inside a close petri dish for 4 hours (Barss, 1968)
5. Weight the leaf disks in order to obtain saturated (turgid) weight.
6. The leaf disks are removed using tweezers from the petri dish, blotted dry on a paper towel and put it in an envelope and oven later. The oven temperature is 85°C for 48 hours
7. After dried, weight the leaf disks in order to obtain the dry weight
8. All the weights should be weighed on an analytical balance to the fourth decimal point and then rounded off the third decimal point
9. RWC can be then calculated using the formula above and fill up Table 2.

Table 1

<table>
<thead>
<tr>
<th>Crop</th>
<th>Hour</th>
<th>Rep</th>
<th>Fresh wt. (g)</th>
<th>Dry wt. (g)</th>
<th>% MC</th>
<th>Ave. of %MC</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ipomea batatas/Amaranthus sp.</em></td>
<td>15</td>
<td>I</td>
<td></td>
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<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>II</td>
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<td></td>
<td>23</td>
<td>I</td>
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</table>

By PEMW for Semester I- 2014/2015
Table 2

<table>
<thead>
<tr>
<th>Crop</th>
<th>Trt (%MC)</th>
<th>Rep</th>
<th>Fresh wt. (g)</th>
<th>Saturated wt. (g)</th>
<th>Dry wt. (g)</th>
<th>RWC</th>
<th>Ave of RWC</th>
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Meeting 3:
b. Water and Plant

Introduction

There are two major ways to move molecules: A. Bulk (or Mass) Flow - is the mass movement of molecules in response to a pressure gradient. The molecules move from high to low pressure, following a pressure gradient. B. Diffusion - the net, random movement of individual molecules from one area to another. The molecules move from high to low concentration, following a concentration gradient. Another way of stating this is that the molecules move from an area of high free energy (higher concentration) to one of low free energy (lower concentration). The net movement stops when a dynamic equilibrium is achieved. Osmosis is a specialized case of diffusion; it represents the diffusion of a solvent (typically water) across a membrane.

Water potential is a measure of the energy state of water. This is a particularly important concept in plant physiology because it determines the direction and movement of water. Water potentials in intact plant tissue are usually negative (because of the large quantities of dissolved solutes in cells). Water always moves from an area of higher water potential to an area of lower water potential. Water potential is affected by two factors: pressure and the amount of solute.

Equation for water potential (must account for the factors that influence the diffusion of water):

\[ \Psi_w = \Psi_p + \Psi_s + \Psi_g \]

Where,
- \( \Psi_w \) = water potential
- \( \Psi_p \) = pressure potential
- \( \Psi_s \) = solute or osmotic potential
- \( \Psi_g \) = gravity potential

Solute (or osmotic) potential (\( \Psi_s \)) is the contribution due to dissolved solutes. Pure water at atmospheric pressure has a solute potential of zero. As solute is added, the value for solute potential becomes more negative. This causes water potential to decrease also. In sum, as solute is added, the water potential of a solution drops, and water will tend to move into the solution. The solute potential of a solution can be calculated with the Van’t Hoff equation:
Ψₚ = - miRT

Where,  
- \( m \) = molality (moles/1000 g)  
- \( i \) = ionization constant (often 1.0)  
- \( R \) = gas constant (0.0831 liter bar/mole K)  
- \( T \) = Temperature in degrees Kelvin (273 + °C of solution)

Pressure (or Pressure Potential; \( \Psi_p \)) - In a plant cell, pressure exerted by the rigid cell wall that limits further water uptake. It is usually positive, although may be negative (tension) as in the xylem. Pressure can be measured with an osmometer. Matric potential is the contribution to water potential due to the force of attraction of water for colloidal, charged surfaces. It is negative because it reduces the ability of water to move. In large volumes of water it is very small and usually ignored. However, it can be very important in the soil, especially when referring to the root/soil interface. Gravity (\( \Psi_g \)) is contributions due to gravity which is usually ignored unless referring to the tops of tall trees.

Experiment: Determination of water potential of plant tissue

Objectives: 1. To determine dynamic equilibrium between potato tissue and solution

Methods

1. Dispense 50 mL of sucrose (0, 0.05, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35 M, molar) into each of eight appropriately-labeled containers. (note: sorbitol, mannitol or polyethylene glycol can be used in place of sucrose).
2. Use a cork borer (0.5 – 1.0 cm) to prepare at least 8 potato cylinders for about 3 -4 cm long. Cut them to the same length with a razor blade (ca. 4.0 cm). Be sure not to include any fragments of the skin. Work quickly to minimize evaporation and keep the tissue wrapped in a moist towel.
3. Weigh all potato cylinders and record your data in Table 1. Immediately after weighted, cut them into slices for about 2.0 mm thick and then place the core slices in one of the beakers. Repeat for all solutions. All the weights should be weighed on an analytical balance to the fourth decimal point and then rounded off the third decimal point
4. Incubate the cores for 1.0 hour. Then remove the tissues, gently blot on paper towels and reweigh. Record your data in Table 1. Examine the slice cores as you weighted them.
Results and Discussions

1. Complete Table 1 (show the calculation). Use the following equation:
   a) to calculate the percent change in weight for each tissue by the following equation:

   \[ \text{% change in weight} = \frac{\text{final wt.} - \text{initial wt.}}{\text{initial wt.}} \times 100 \]

   b) to calculate osmotic potential for all sucrose concentration by using the following equation:

   \[ \Psi_s = -m_i \text{RT} \]

2. Plot % change in weight (y axis) vs. sucrose concentration (M, molar) and osmotic potential (x axis). Draw the best fit line for your data.

3. From the graph, determine the concentration of the sucrose solution in which there was no net weight gain (i.e., % change = 0). At this point, the water potential of the solution equals the osmotic potential of the potato tissue.

<table>
<thead>
<tr>
<th>Table 1: Change in weight of potato cores incubated in sucrose solutions</th>
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<tbody>
<tr>
<td>[Sucrose] (molality)</td>
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<td>----------------------</td>
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<tr>
<td>0</td>
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<td>0.35</td>
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</table>
After calculate one $\Psi$ for one concentration, $\Psi$ for other concentration can be calculated with formula shown below:

\[
\frac{M_1}{\Psi_1} = \frac{M_2}{\Psi_2}
\]

4. How to prepare 1 litre of 0.6 molar sucrose? What is the different between this solution with sucrose of 0.6 molal?

5. Do the cores show various degrees of turgor? Explain
Meeting 3:
c. Presentation

Students have to present their Mind Mapping Assignment.