Plant Physiology and Biotechnology
Biology 3470
Winter 2006 Lab and Course Outline

Instructor: D. Law, CB4018
phone 343-8277, dlaw@lakeheadu.ca
Office hours Friday 9:30 to 10:30 AM, otherwise by appointment.

Class location: AT2019, 12:30 – 1:30 pm, Tuesdays and Thursdays

Lab location: CB3010B, 8:30 AM-11:30 AM, Wednesday

Course website: http://flash.lakeheadu.ca/~dlaw/3470.html
The website will have the latest course updates and information.

Course objectives:
To introduce concepts about plant physiology, biochemistry and biotechnology at an advanced level to enable a practical understanding of the cardinal importance of plant metabolism in the biosphere, and how it may be manipulated through genetic engineering to make products of high agronomic value.

Textbook:
Hopkins WG, Huner NPA (2004) Introduction to plant physiology, 3rd ed., Wiley. (available at bookstore or from amazon.ca)
INTRODUCTION

Welcome to the exciting world of plant science! By the end of this course, it is hoped that you will recognize that the unique metabolism of plants raises many interesting research questions. Understanding how plants grow and reproduce has profound consequences for better comprehending how primary elements cycle through the biosphere. Plants are required for conversion of the carbon and nitrogen in the atmosphere to metabolically useful (and tasty) molecules for animals, bacteria and fungi.

Some of the labs in this course can be finished within the three hours allocated, but others will require some additional time investment on your part (see the Lab Timetable). This is mainly due to the long developmental times of plants. To lessen the demands on your time, you will work in small groups of two to three, allowing you to share some of the tasks. Hopefully, doing “hands-on” science will provide you with enough inspiration to put in your best effort in order to get meaningful data for the whole group.

The main goals of these exercises are not only to give you some laboratory experience, but also to give you the opportunity to interpret your own data and to learn how to communicate your results and research ideas to others. In this sense, even the simplest of experiments will serve this purpose.

To help you with the preparation of many of the reports, references have been provided that are directly, or marginally, related to the labs. These will be available in the library in the journal stacks, providing you with an opportunity to do literature searches. Ideally, when you write up each lab, you will have used these lit search skills to find and use relevant, recent literature (note that marks are allocated to the reference section for each lab). The two best sources are:

- Agricola (http://www.agricola.nal.gov)

These US government electronic resources together list every plant (and animal) based research paper published in the last 40 years. Agricola is especially useful for searching on plant based topics as PubMed only started cataloguing plant science journals within the last eight years or so.

Work hard and have fun!
<table>
<thead>
<tr>
<th>Lab # (Wed.)</th>
<th>Lab name</th>
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<td>1a</td>
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<td>1b</td>
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<td>Gibberellic acid effect on α-amylase secretion in barley seeds I</td>
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<td>4b</td>
<td>Plant nutrition II</td>
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<td>STUDY BREAK</td>
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<td>3b</td>
<td>Gibberellic acid effect on α-amylase secretion in barley seeds II</td>
<td>1-Mar</td>
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<td></td>
<td>Paper discussion I: heterosis</td>
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<tr>
<td>6a</td>
<td>Effect of PGRs on pea seedlings I</td>
<td>8-Mar</td>
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<tr>
<td>1c</td>
<td>Tissue culture III</td>
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<tr>
<td>7</td>
<td>Water relations</td>
<td>15-Mar</td>
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<td>Effect of PGRs on pea seedlings II</td>
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<td>22-Mar</td>
<td>Paper discussion II: fruit size and shape</td>
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<td>29-Mar</td>
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<td>Gibberellic acid effect on α-amylase secretion in barley seeds II</td>
<td>22-Feb</td>
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<td>3-Mar</td>
<td>Lab 4: Plant nutrition</td>
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<tr>
<td>10-Mar</td>
<td>Lab 3: Gibberellic acid and α-amylase secretion</td>
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<td>7-Mar</td>
<td>Lab 1: Tissue culture (large)</td>
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<tr>
<td>24-Mar</td>
<td>Lab 7: Water relations</td>
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<tr>
<td>31-Mar</td>
<td>Lab 6: PGRs</td>
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LAB REPORTS:

1. Overall summary

Most labs do not give complete results on the day of the lab. This is because plants need time to grow and develop, and thus to give results from experiments. This means that most of the labs will not be finished until the second half of the term, and most reports will be due at that time. This does not mean that much of the work (research, writing of the intro/literature review, anticipating and preformatting results sections [will you present tables, graphs, or some other type of figures?], etc.) cannot be done beforehand!

The reports will take the form of a brief scientific paper. They will consist of these main sections: Abstract, Introduction, Materials and Methods, Results, Discussion and References. In the Introduction, review the reasons for doing the experiment and the literature which stimulated you to do the experiment (try to pretend, anyway). In the Results, simply describe the data you have obtained, i.e., direct the reader's attention to any Figures or Tables you have. Use only one graph (called a Figure) per set of data. Don't report both raw numbers and graphs. If there is no reasonable way to prepare a figure, summarize the data and place it in a numbered table (Table 1, etc.). Always present some statistical analysis of the numbers).

Offer explanations and interpretations of the data in the Discussion. Concentrate on the biological responses of your experimental system, don't get bogged down discussing the methodology or instrumentation, unless these are the main topic or are directly relevant to the understanding of the biological response. An especially important aspect of the Discussion is being able to relate your own observations to those of others, i.e., you MUST read and refer to the work of others. Suggest further experiments that would strengthen your hypothesis. Make sure that you relate your work to previous observations in terms of relative probability of being close to the truth, not in absolutes such as: "here we prove the existence of vegetable soul". Try instead: "since our treated group of plants responded positively to sound, we suggest that electrochemical events took place in the plant tissue, which may be akin to those observed in higher animals (Ref.)". It may seem like being too wishy-washy, but in science it usually takes many little truths to make one big speculation (not necessarily a big truth).

The Abstract is the most difficult portion of any report, write it last! In one sentence each, describe why, how, and what of the experiment, i.e., the reasons for doing the work, the basic method employed and the findings. The Materials and Methods section describe concisely what was done and with what materials (sources of instruments and chemicals in brackets!!). Make sure that you understand what you were doing, as you may be asked related questions in the exams. In the report, only refer to the sources of your methods and briefly describe any deviations from the lab manual protocols. References should be presented in the style of the journal Plant Physiology.

2. Style

Use short, concise sentences. Remember that you are not writing a novel or even an English essay. The sentences should be to the point, but well (logically) joined together. Use the
active voice in a narrative style; telegraphic statements of facts should be avoided. Use the present tense and active voice if possible and don't change the tenses. Refrain from using emotional words such as "gorgeous data" or "pitiful statistics" (these phrases are generally the privilege of reviewers and critics!!). Allow some of your personality to come through (the famous phrase from Watson and Crick's paper on DNA structure "it has not escaped our attention..." is a classic).

To reiterate once more: pay attention to the contents appropriate for each section of the report. If you are in doubt as to what is expected, look at some papers published in the journal Plant Physiology. Read the book Writing to Learn Biology by Randy Moore, it is the standard writing book adopted by our Biology Department.

3. Marking

The evaluation of your type-written reports will be based on the points raised above. Emphasis will be placed on the quality of the data (i.e. the care which went into the gathering of the data, including preparation for the lab), the quality and clarity of data presentation (marks will be deducted for presenting the same data more than once!) and most importantly, your own data interpretation and evaluation of published reports in the Discussion. The heavy marks emphasis on the lab portion of the course (50%) is to give you a lot of opportunity to learn how to prepare scientific reports. The details of reporting style and report organization may differ in other scientific disciplines, but the basic principles should be useful to those of you expecting to write technical reports of any kind in your future careers.

In order to maintain some degree of fairness to those who hand in the reports in on time, **10% of the lab report mark will be deducted for each day your report is late.**

As indicated above, the lab reports are structured as follows:

- **Mini-reports**

  Each one out of four; each worth 4% of final mark. You will submit six mini reports, but the lowest mark will be discarded.

  Maximum of five double spaced pages per report plus figure(s); submit in Arial 12 pt font only.

  1. Abstract /0.5
  2. Introduction /0.5
  3. Materials and methods /0.5
  4. Results /1
  5. Discussion /1
  6. References /0.5
• Large report

Marked out of 10 (worth 10% of final mark). Maximum of ten double spaced pages (plus figures). Sections as above, with an added Abstract. Note from the marking scheme that more emphasis is placed on results and discussion sections. Marking scheme:

1. Abstract /1
2. Introduction /1
3. Materials and methods /1
4. Results /3 (includes figures)
5. Discussion /3
6. References /1

As stated above, all reports are due on the Friday one week after completion of the corresponding lab.

PRELAB QUIZZES / ABSTRACTS

The demonstrator will ask one or two questions related to the lab you are about to perform. The purpose is to make certain you understand what you are about to accomplish in the lab exercises. The best way to prepare for these quizzes is to make sure that you have read and understood the lab material prior to coming to class.

Alternatively, you may be asked to submit a short review of a paper that will be discussed by the class during the lab period.

LAB EXAM

This will contain material from the labs. It may indirectly include lecture material as it pertains to understanding the labs (thus, going to lecture should help greatly in doing well in the labs!). However, the lab exam will focus on the whats, hows and whys of the labs, and what your experiments hoped to find.
Marking scheme for course

Half marks from lab component, half from lecture component, allocated as follows:

- 4 minireports @ 5% each......................20%
- 1 large report.................................10%
- 8 prelab quizzes.........................5%
- lab exam.........................................10%  Last lab period, Wednesday, March 29
- participation and hand-in
  for paper discussions...................5%
- course midterm............................20%  In-class, Thurs. Feb. 16
- course final exam..........................30%

TOTAL 100%

On reserve

The following plant science textbooks have been placed on reserve in the library for this course. The Hopkins and Huner text is intended as a general introductory textbook and can lack detail in certain areas. These texts are useful for their depth on certain plant science topics.

1. Plant physiology, Hans Mohr. QK 711.2 M6413 1995

Note also that I have many other texts that I would be willing to lend out; see me to discuss.
Lecture summary (preliminary and subject to change)

<table>
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<th>Topic</th>
<th>Text ref (chapter/section)</th>
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<td>Introduction</td>
<td>1</td>
</tr>
<tr>
<td>2 Thurs Jan 5</td>
<td>Chemical reactions and enzymes</td>
<td>2.1 and 2.2</td>
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<tr>
<td>3 Tue Jan 10</td>
<td>Water and water relations</td>
<td>10; 11.1 to 11.4</td>
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<td>4 Thurs Jan 12</td>
<td>Xylem</td>
<td>1.7.5; 11.5 to end chp 11</td>
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<td>5 Tue Jan 17</td>
<td>Phloem transport</td>
<td>1.7.5; 6.3 to 6.9</td>
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<td>6 Thurs Jan 19</td>
<td>Phloem, continued</td>
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<tr>
<td>7 Tue Jan 24</td>
<td>Phytochrome</td>
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<tr>
<td>8 Thurs Jan 26</td>
<td>Photosynthesis - light reactions</td>
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<td>Photosynthesis- dark reactions</td>
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<td>Starch and sugar synthesis</td>
<td>6.1, 6.2</td>
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<tr>
<td>11 Tue Feb 7</td>
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<td>7 and 9</td>
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<tr>
<td>12 Thurs Feb 9</td>
<td>N assimilation</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>Tue Feb 14</td>
<td>Finish N assimilation and review</td>
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<td></td>
<td>Thurs Feb 16</td>
<td>Mid-term (in class)</td>
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<td>Mineral nutrition</td>
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<td>Stomatal regulation</td>
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<td>16 Thurs Mar 9</td>
<td>Phytohormones-I</td>
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<td>Genes and gene regulation</td>
<td>on dev’t &amp; growth: 14.1-14.2; controlled by PGRs: 14.4-14.5</td>
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<tr>
<td>19 Tue Mar 21</td>
<td>Crop physiology: feed the world</td>
<td>(supplemental material)</td>
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<td>20 Thurs Mar 23</td>
<td>Stress responses</td>
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<tr>
<td>21 Tue Mar 28</td>
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<tr>
<td>22 Thurs Mar 30</td>
<td>Finish Plant biotech and review</td>
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Feb 20-24 WINTER BREAK

| 13 Tue Feb 28 | Mineral nutrition                         | 12                          |
| 14 Thurs Mar 2 | Signal Transduction & Tropisms             | 14.3; 18                    |
| 15 Tue Mar 7 | Stomatal regulation                        | 5.1 to 5.4                  |
| 16 Thurs Mar 9 | Phytohormones-I                           | 16                          |
| 17 Tue Mar 14 | Phytohormones-II                          |                             |
| 18 Thurs Mar 16 | Genes and gene regulation                 | on dev’t & growth: 14.1-14.2; controlled by PGRs: 14.4-14.5 |
| 19 Tue Mar 21 | Crop physiology: feed the world            | (supplemental material)     |
| 20 Thurs Mar 23 | Stress responses                           | 21                          |
| 21 Tue Mar 28 | Plant biotech                              | 23                          |
| 22 Thurs Mar 30 | Finish Plant biotech and review            |                             |

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<td>Gibberellic acid and amylase</td>
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<td>7</td>
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LAB 1: TISSUE CULTURE (large report)

Week 1: 11-Jan  
Week 5 – after 4 week growth period (8 Feb)  
Week 9 - after further 4 week growth period (8 Mar)

1. Purpose

To use classical tissue culture systems – potato tuber and carrot root - to investigate the role of plant growth regulators in organogenesis.

2. Introduction:

Cells in the organs of multicellular organisms are of different types because they have differentiated to perform specific functions. These specialized cell types arise from stem cells. Stem cells are totipotent, meaning that given the appropriate positional and hormonal cues, they may differentiate into all the cell types present in an organism. In animal cells, de-differentiation of cells back into stem cells is not possible because the developmental program prevents this.

By contrast, cells taken from some parts of plants are able to revert back to a totipotent state. When excised plant organs are placed on media containing an appropriate balance of plant growth regulators (PGRs), their component cells form an undifferentiated mass called a callus. For example, explants of carrot root and potato tuber will form callus when placed on media containing both kinetin and auxin. Cells in the callus will divide in tissue culture. By manipulating the PGR concentration of the medium, callus cells can be stimulated to produce shoots and roots, and thus to regenerate new plants. The specific conditions leading to these de- and re-differentiation steps will be studied in this lab exercise.

What is plant tissue culture used for? It is a key technique that allows the production of transgenic plants. Agricultural biotechnology involves the production of crop plant seed that possesses high-value traits. These include herbicide resistance (e.g., Roundup Ready from Monsanto) and resistance to pest attack (biotic stress, such as herbivory/insect damage). Biotech companies are now actively researching and marketing genes that allow resistance to abiotic stresses such as drought, salt and temperature. All of these desirable features are contained on genes that were found in other organisms (both plant and non-plant), and must be introduced into crop plants.

Callus tissue is especially amenable to the uptake of foreign DNA, either through biolistics (gene gun) or via transformation using the bacterium Agrobacterium tumefaciens (an organism we will study in a later lab). Plant tissue culture techniques have been adapted for use in many species, but some plants (e.g., monocots/grasses) have proved more difficult to manipulate to produce callus and regenerate plants. Once callus is manipulated genetically in vitro to introduce foreign DNA, it is hoped that these cells will incorporate the genes into their genomic DNA. Once new plants are regenerated from this callus, plants containing the foreign gene (transgene) can be selected, grown to maturity and their seeds collected. These transgenic seeds should express the novel phenotype in a new generation of plants (R1). This lab will give you a taste of the basic approach used to produce callus and regenerate plants using plant tissue culture.
3. Materials:

Petri dishes
Sterile water
1% bleach solution (a 20% dilution of Javex household bleach in water)
Sterile transfer box
Scalpel and forceps with a set up for flame sterilization
Carrot root and potato tubers
Cork borer (size 3)
Sterile filter paper

4. Procedures:

IMPORTANT: Use sterile technique. This will be demonstrated. This involves
- not waving hands over open flasks and plates
- washing hands
- keeping work area clean
- using sterile (laminar) hood for manipulations

WEEK 1: Induce dedifferentiation of plant tissue (4 weeks).

First make the stocks of the growth regulators. These recipes make 1 mg / mL stocks:

- **Auxin (IAA):** dissolve 10 mg in 0.5 mL 1-M NaOH, then add 9.5 mL H2O.
- **Kinetin:** dissolve 10 mg in 1 mL 1-M HCl, swirl/heat to dissolve, then add H2O to 10 mL total.
- Filter-sterilize these into sterile 15-mL tubes. Store at -20°C.

Now prepare callus induction medium. This is based on Turhan (2004):

- MS (Murashige and Skoog) salts and vitamins
- Sucrose 30 g/L
- Kinetin 0.5 mg/L
- IAA (indole-3-acetic acid; auxin) 5 mg/L
- Agar 12 g/L
- pH 5.8

Prepare 500 mL of the callus induction medium in a 2-L erlenmeyer flask. It must then be sterilized by autoclaving (this will be demonstrated), let cool to a temperature where medium is not hot to touch, and dispensed into Petri dishes to a depth of about 4 mm.

You must excise and sterilize carrot root and potato tuber explants. Do this in a sterile hood (Malek lab) while the medium is autoclaving and cooling.
1. Cut out sample cylinders with cork borer (~3 mm diameter).
2. Surface sterilize in 1% sodium hypochlorite 5-15 min (stop when carrot starts to lose surface colour).
3. Rinse in sterile water in petri dish. Repeat with fresh sterile water.
4. Blot gently on sterile filter paper
5. Cut in ~3 mm thick sections with sterile scalpel blade.

*It is very important to use sterile technique for the following steps!* Otherwise your callus induction medium will be mostly fungus from the air and your hands!

6. Using sterile alcohol dipped flamed forceps, place tissue sections on gelled callus induction medium (4 sections/plate).
7. Wrap in parafilm, place in greenhouse in diffuse light right side up. Observe carefully over the next several days for excessive moisture build-up. Remove parafilm if this occurs.
8. Continue to monitor for signs of contamination or drying out. Reparafilm if necessary.

**WEEK 2: Induction of organogenesis in 4-week-old callus**

By now the tissue should have dedifferentiated into callus.

Now we will induce shoot and root growth by manipulating the plant growth regulator concentration in the medium.

Again, you will make MS medium, this time as follows.

Make 2 L of MS base medium. This will be used to make 100 mL of each treatment medium (for 4 plates/treatment).
Add the growth regulators to each dish as follows in the sterile hood. Note the dish numbers in bold; label the bottoms and tops of your dishes with these numbers.

<table>
<thead>
<tr>
<th>Kinetin concentration (mg/L)</th>
<th>0</th>
<th>0.5</th>
<th>1</th>
<th>2</th>
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<tbody>
<tr>
<td>0</td>
<td>1: 0 ul IAA 0 ul kinetin</td>
<td>2: 12.5 ul IAA 0 ul kinetin</td>
<td>3: 25 ul IAA 0 ul kinetin</td>
<td>4: 50 ul IAA 0 ul kinetin</td>
</tr>
<tr>
<td>0.5</td>
<td>5: 0 ul IAA 12.5 ul kinetin</td>
<td>6: 12.5 ul IAA 12.5 ul kinetin</td>
<td>7: 25 ul IAA 12.5 ul kinetin</td>
<td>8: 50 ul IAA 12.5 ul kinetin</td>
</tr>
<tr>
<td>2</td>
<td>13: 0 ul IAA 50 ul kinetin</td>
<td>14: 12.5 ul IAA 50 ul kinetin</td>
<td>15: 25 ul IAA 50 ul kinetin</td>
<td>16: 50 ul IAA 50 ul kinetin</td>
</tr>
</tbody>
</table>

Now add 25 mL hot medium/dish (not boiling but ~55°C, so that you can comfortably hold the flask in your bare hand), dissolve the PGRs by
- Gently pushing dish away from and towards you on the laminar hood surface 3X
- Then left to right 3X
- Then swirl clockwise 3X
- Then swirl counterclockwise 3X.

Let stand in hood with lids open to solidify. After gelation occurs, the medium is ready for receiving tissue.

MATERIALS:
- 16 petri dishes per group (1 dish / treatment, x 16 treatments) x # of student groups
- 0.8 % (w/v) agar
- 250 mL and 1 L flasks for media preparation
- Sterile water
- Stock solutions of kinetin (for final concentrations of 0-2 mg/L) and indole-3-acetic acid (IAA, final concentration 0-2 mg/L)
- Sterile transfer hood
- Autoclave
- Scalpel and forceps with a setup for flame sterilization
- Callus tissue

1. Clean sterile transfer box, tools and your hands.
2. Aseptically cut small pieces of potato and carrot callus in Petri dishes.
3. Place bits of callus into new Petri dishes with media - how many will depend on the amount of callus you get from Week 1.
4. Seal dishes with parafilm, wrap in foil.
5. Culture under constant temperature in the dark - some groups may investigate the effect of light period and intensity on the organogenic process.

6. Make weekly observations (notes!!)

**WEEK 3: Determine effect of plant growth regulators on organogenesis.**

After 4-6 weeks remove callus, determine fresh weight and number of roots or shoots. Report your observations and average numbers for each treatment.

As with all of the lab writeups, use statistics whenever you can to help interpret your data and determine its significance (use basic methodology you learned last year in 2nd year stats, e.g., mean +/- standard deviation).

**5. Report:**

One of the original reports by F. Skoog came out in Symp. Soc. Exp. Biol. II, 1957, and many reports have come out since. Practice your literature searching skills on this one. This lab report is the only large report due (see course intro for format). Make the best of your time in preparing it.

LAB 2: Factors affecting plant yield

WEEK 1: 18-Jan
WEEK 2: 8-Feb, after 3 week growth period

1. Purpose

To determine how plant growth is affected by environmental factors.

2. Introduction

A common measure of the performance of plants in farmers’ fields is their yield. In agronomic (the economics of agriculture) terms, this generally refers to the amount of material in crop plants harvested that is usable by humans, either as food (as is or extracted for starch, sugar or oil), animal feed, or other uses such as textile production. The vast majority of crop plants grown in North America and Europe are elite high-yielding varieties that have been selectively bred for high product yield. The fortunes of seed companies, such as Pioneer Hi-Bred, Monsanto and Syngenta, are directly linked to the quality of seed that they sell to farmers: the higher its yield (among other factors), the more popular and financially successful it is likely to become. This is why seed companies large and small invest significant amounts of money in germplasm (high-yielding parental plant lines) development. They are always searching for a new combination of alleles that results in improved agronomic traits.

The amount of biomass produced by growing plants and its partitioning between photosynthetic tissue and seed are affected by growing conditions. Obvious elements critical to plant life are water, sunlight and temperature, but ideal environmental conditions vary between species and even between cultivars and ecotypes of a single species.

In this lab, you will examine how several environmental factors impact plant growth and development, and compare their effect on a monocotyledonous C4 species (Zea mays, maize or corn) and a dicotyledonous C3 plant (Glycine max, or pea).

3. Materials

You will be provided with large (6” diameter) pots of peas and maize seedlings, 7 days old, grown in coarse vermiculite. The experiments involve comparing treatments between species, or within species under different growing regimes. Standard growing conditions in the greenhouse are ~27 C daytime/~19 C night, with a 16-h photoperiod. Different areas of the greenhouse receive different amounts of light. This will be exploited in this series of experiments. You can measure light intensity with a light meter (which will be provided and demonstrated).
4. Procedures

**Factor 1: Fertilization and Rhizobium inoculation.**

Rhizobia spp. (species) are bacteria that fix nitrogen from the soil. Farmers spray a solution of these bacteria on the soil during planting. Look in your text to determine how they aid in plant growth. In brief, the bacteria are taken up by the roots of certain plant species and initiate the growth of nodules just below the soil surface, where they enter into a symbiotic nitrogen-fixing relationship with the plant.

Nodulation is an evolutionary response to the often limited availability of certain mineral nutrients in a plant’s environment. This is either due to the mineral being absent from the soil or to constraints on the plant’s ability to make use of the mineral, due to uptake, solubility or toxicity issues.

Small pots of corn and peas have either been inoculated with Rhizobia upon sowing, or have not (4 pots total). None of these plants were fertilized after planting.

4 additional pots (2 of corn and 2 of peas) have been treated or not treated with Rhizobia in the same manner as above. These plants have been fertilized with a dilute solution (5ml -1tsp per watering container) of general purpose fertilizer (20-20-20) regularly during growth.

Thin seedlings in all pots to the 6 largest/pot.

Place one pot of each species and treatment (8 pots total) in the same place in the greenhouse, and monitor growth over the next 4 weeks (take notes). Irrigate regularly with the appropriate treatment - fertilizer solution or water during this time period.

At the end of the 4 week period, determine how the treatments affected yield by gently uprooting the plants, rinsing them in water, patting dry, and weighing all the plants from one pot together. Report average plant fresh weight +/- SD (per plant: divide total weight by the number of plants!). Dry the seedlings in an oven at 80C overnight to get dry weight, Report average plant dry weight +/- SD. Also, count the number of nodules per pot and report as average number per plant +/- SD.

Some questions to address in your report include:

How does Rhizobium work to increase plant growth? Do you expect the same response from pea and corn? Hint: what are the family names of both species? What happens to yield and nodule number when you provide fertilizer to the plants?

**Factor 2: Light level.**

Pots of peas and corn (sown and thinned as above) will be grown in the greenhouse. One pot of each will be grown under high light (16-hour photoperiod with supplemental sodium lighting), and another set under an empty bench. These plants will be regularly fertilized using the same fertilizer and strength after germination. Use the light meter to measure the light intensity at the two locations, ideally at local noon to give a maximum “natural” value (~12:30 pm).

Repeat yield determination after 4 weeks for the plants grown in different light levels as for
Rhizobium inoculation. On a molecular level, can you suggest some ways that the photosynthetic apparatus of the plants grown at the two light regimes differ? What would happen if you reversed the light regimes after a few weeks? Is there more of a difference between pea and corn?

**Factor 3: Light quality.**

As you have heard in lecture, the photosynthetic machinery of plants is optimized to absorb light of particular wavelengths. This portion of the lab will be a practical demonstration of this fact. Pots of pea and corn will be grown under different coloured filters at a high light regime. The colours used will be

- Red
- Blue
- Green
- Clear

Carefully measure the heights of the seedlings in each pot. Place one pot of each species of fertilized seedling (sown and thinned as described previously) in one of the boxes under each filter at the high light regime. Note the effect of yield after 4 weeks as for Rhizobium inoculation.

**5. Report**

Explain your results on a molecular level. What might be another reason for the difference (if any) between treatments? Why are the seedlings fertilized?

What is the purpose of the clear filter grown plants?
LAB 3: GIBBERELLIC ACID AND STARCH HYDROLYSIS IN GERMINATING BARLEY SEEDS

WEEK 1: 15-Feb
WEEK 2: 1-Mar

1. Purpose:

To study the role of the embryo and gibberellic acid in barley endosperm starch hydrolysis.

2. Introduction:

The observation that embryo regulates the expression of the alpha-amylase gene in the barley aleurone via the release of gibberellic acid has become one of the classic observations of plant physiology. In this lab you will repeat some of the observations made by the early investigators in this field and reinforce the basics of aseptic technique with another plant system.

3. Materials:

- 8 sterilized petri dishes (9-cm diameter) with lids
- 50 barley seeds
- fine-mesh screen or cheesecloth large enough to cover mouth of 100-ml or 250-ml beaker with elastic
- 100- or 250-ml beaker in which seeds will be surface sterilized
- 125-ml of sterile distilled water in 250-ml flask
- 100 ml of 95% ethanol plus burner
- scalpel with sharp blade
- forceps
- pipettors
- sterile pipette tips, both 10-200 µL (yellow) and 200-1000 µL (blue)
- household bleach
- graduated cylinder
- starch agar culture medium (7 tubes, 13 ml media/tube)
- 0.3 mg/ml chloramphenicol solution
- 1 µM GA₃ solution
- 0.15 mg/ml cyclohexamide solution
- sterile distilled water
- iodine reagent

4. Procedures:

WEEK 1: 15-Feb
In this experiment it is essential to use sterile techniques whenever possible to prevent contamination of your agar-containing test plates by microorganisms, especially fungi and fungal spores (they are all over your hands and in the environment!). Begin by washing your working area with water, wipe it with a paper towel, then wipe it again with a paper towel soaked in 95% ethanol.

You will be provided with 7 culture tubes each containing 13 ml of autoclaved and still hot, unsolidified starch agar solution. All such solutions contain 1% bacto-agar and 2% soluble potato starch. These tubes are plugged with cotton to prevent contamination from air-borne microorganisms.

Number seven of your petri dishes from 1 to 7, marking both the lid and base of each dish and taking care to keep the lids on while marking them. Using a pipettor with a sterile tip, add 1.0 ml of 0.3 mg/ml chloramphenicol (chloromycetin) solution to each of your seven petri dishes. Remove the lid of each dish only enough to the dispense the solution, then replace it. Discard the tip.

Now add the additional components to the dishes as follows:

<table>
<thead>
<tr>
<th>Petri dish No.</th>
<th>Sterile distilled water</th>
<th>Gibberelic acid 1 μM</th>
<th>Cycloheximide 0.15 mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.0 ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1.0 ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>1.0 ml</td>
<td>10 μl</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1.0 ml</td>
<td>100 μl</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>100 μl</td>
<td>1.0 ml</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>1.0 ml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>1.0 ml</td>
<td>1.0 ml</td>
<td></td>
</tr>
</tbody>
</table>

Swirl the covered dishes gently to mix the components. Now add a cooling (but still at 50°C or warmer) starch agar to each dish, quickly repacing lids. To thoroughly mix all materials present in the dishes, use the following technique: Swirl each dish six times clockwise, six times counterclockwise, six times forward and back, then six times left to right. Spread out the dishes on your bench top so that the agar will rapidly cool and solidify.

Place 50 barley seeds in a 100-ml or 250-ml beaker and cover them with 20-ml of undiluted household bleach (about 5 percent NaOC1, sodium hypochlorite) to kill microorganisms on their surfaces. Swirl the flask occasionally during a period of 10 to 20 min. Cover the beaker with a screen or cheesecloth to prevent loss of the seeds, then decant the bleach, discarding it. Rinse the seeds with 10 separate portions of sterile water, using approximately 10 ml each
time, or until no more bleach odour remains on the seeds. Save enough sterile water so that 10 ml may be used in the petri dish in which the seeds are to be cut.

Tap at least 6 of the seeds into the remaining unused petri dish, add about 10 ml sterile water, and replace the lid. Dip the blade of the scalpel in 95% ethanol, burn off the alcohol in a flame, and then partly remove the lid of the petri dish to minimally expose the seeds. Cut 6 of the seeds transversely across their middle. While cutting them group the half-seeds still containing embryos separately from those without embryos.

Using alcohol-flamed forceps transfer 6 embryo-containing half-seeds into dish No. 1, spacing them evenly around the plate about 2 cm from the edge. Force the cut ends solidly down into the agar. Replace the petri dish lid immediately. Similarly transfer the six non-embryo-containing half-seeds into dish No. 2. Again alcohol-flame the scalpel and cut another group of seeds, discarding the embryo-containing halves and adding those without embryos to dish No. 3. Repeat until all of the dishes also contain six half-seeds without embryos.

Place the dishes in a convenient place at room temperature (20° to 23°C) for five days. (After five days they may be refrigerated by the instructor until the next laboratory period.) During this incubation period amylase secreted by the half seeds will diffuse outwardly and digest starch.

in the agar.
WEEK 2 (1-Mar):

Starch digestion can be detected by looking at diffuse light through the plates. To make the digested starch more visible, the plates can be stained with iodine. Remove the lids and spray the dishes with a dilute iodine reagent that forms a blue color with undigested starch (1.0 gram I₂ and 2.0 grams KI in 1 liter of H₂O). Alternatively, the I₂KI may be poured on the agar surfaces, then poured off after 2 or 3 min. Note the unstained halos around the half seeds that have secreted α-amylase. Measure and compute (in centimeters) the average diameter of halos in each dish; list data in the report sheet.

5. Report:

Include answers to the following questions in your report.

1. Did the non-embryo-containing half-seeds secrete as much amylase as the embryo halves?
   Why?

2. Describe the effect of various amounts of GA₃ upon starch digestion.

3. What was the function of chloramphenicol in the petri dishes?

4. What was the effect of cycloheximide (actidione) upon starch digestion? What does this suggest concerning the mechanism of α-amylase production in the aleurone layer?

5. Concerning the known antibiotic mechanisms of action of chloramphenicol and cycloheximide, why should the former not prevent any α-amylase production?

References:


LAB 4: Plant nutrition

Week 1: 1-Feb
Week 2: 15-Feb

Introduction:

All of us water our house plants once in awhile; some of the more vigilant notice that if we exclude fertilizer that the growth rate ceases after a time and that the plants begin to look unhealthy. It is interesting to consider why fertilizers such as “MiracleGro” are so essential for the support of plant growth. In the natural environment, plants have access to macro and micronutrients in the soil, but one or more of these are often limiting to maximum yield. Thus, the “green revolution” of the 1950s and 1960s involved application of “NPK” fertilizers containing enough nitrogen, phosphorus, and potassium to overcome soil deficiencies of these elements. Quite often, the form (ionization state) of the fertilizers available is just as important; the bioavailability of critical elements can be limited in soils possessing extreme pH values and is affected by interactions with other elements.

In this lab, you will test how the growth of tomato plants is affected by the minerals supplied to them.

Plants in this lab will be grown in solution, also known as hydroponically. These are the growth conditions of choice for large scale vegetable (e.g., lettuce) production operations (recently featured on How It’s Made on the Discovery Channel), and also for illegal drug production in grow houses (mostly for Cannabis production). Large scale hydroponic plant production necessitates large amounts of power usage for water circulation, lighting and heating. These energy charges are the bane of commercial greenhouses and also make it easier to detect grow ops in residential neighborhoods.

Procedures:

WEEK 1 (1-Feb):

You will test 6 nutrition regimes on the growth of tomato plants:

1. Complete
2. water only
3. minus N
4. minus Ca
5. minus P
6. minus Fe

This entails making five different nutrient solutions. To do this, use stock solutions that have been provided for you. All had their pH adjusted to 6 to 6.5 after preparation, resulting in nutrient media that have a constant pH. This is essential for optimal growth.

All solutions (except 2 and 6: check carefully!) contain two extra solutions that have been made for you as follows:
A. Micronutrients:

<table>
<thead>
<tr>
<th></th>
<th>g/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>CuCl₂</td>
<td>0.1</td>
</tr>
<tr>
<td>H₃BO₃</td>
<td>3.0</td>
</tr>
<tr>
<td>H₂MoO₄·H₂O</td>
<td>0.05</td>
</tr>
<tr>
<td>MnCl₂·4H₂O</td>
<td>0.05</td>
</tr>
<tr>
<td>ZnSO₄</td>
<td>0.1</td>
</tr>
</tbody>
</table>

Use 1 mL / L nutrient solution.

B. Iron:

Ferrous acetate  30 g/L
To make up the iron stock, take 26.1 g EDTA and dissolve in 286 ml water that has ~19 g KOH. Then dissolve 24.9 g FeSO₄·7H₂O in ~ 500 ml water. Slowly add the iron sulfate solution to the potassium EDTA solution and aerate this solution overnight with stirring. The pH rises to about 7.1 and the solution is wine red and very little precipitation occurs. Make to 1 liter final volume and store in a bottle covered with foil (dark).

Use 0.25 mL / L nutrient solution.

Make up 1 L of nutrient solution for each group according to the following table:

<table>
<thead>
<tr>
<th>Nutrient stock</th>
<th>Vol per L (mL)</th>
<th>complete</th>
<th>H₂O</th>
<th>Minus N</th>
<th>Minus Ca</th>
<th>Minus Fe</th>
<th>Minus P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. KNO₃</td>
<td>6</td>
<td>X</td>
<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>2. MgSO₄</td>
<td>2</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>3. Ca acetate</td>
<td>4</td>
<td>X</td>
<td></td>
<td>X</td>
<td></td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>4. KPi</td>
<td>1</td>
<td>X</td>
<td></td>
<td>X</td>
<td>X</td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>5. Iron</td>
<td>0.25</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
<td>X</td>
</tr>
<tr>
<td>6. Micronutrients</td>
<td>1</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
</tbody>
</table>

**NOTE:** Add the concentrated stocks to a large volume of deionized water (from the white tap). Otherwise they will precipitate out of solution.

The plant material used is 8-d-old tomato seedlings. Carefully remove them from the vermiculite and gently rinse off their roots. Fit the roots through the central hole in a jar lid, then carefully place a foam plug in the hole to hold the plant upright. Fill the jar with the appropriate nutrient solution. Screw the lid with plant onto the jar, and place in the light. The light regime will be a 16-h photoperiod (lights on 6AM – 10 PM).

The root systems must be aerated; otherwise the plants will die from anoxia (lack of oxygen for respiration). Therefore, fit a pasteur pipette attached to an air source into the smaller hole in the lids, and bubble air slowly through the solution. Once the air rate is set, cover
the glass part of the pots with foil to exclude light and prevent algae growth.

Come in twice a week to check the nutrient solution level in the pots and top up as needed. If you do not arrange to do this among your group, the solutions will evaporate and the plants in your experiment will die.

When you top up the nutrient solutions, also take height measurements of your plants from the jar lid to the tip of the stem. Also make qualitative observations about the appearance of your plants.

WEEK 2 (15-Feb):

Remove the plants from the pots. Pat the roots dry. Weigh the whole plant, then sever the shoot from the root and weigh each separately. This is the wet weight. We will then oven dry your samples so that dry weights (without water) can be obtained. These are much more informative about effects of the treatmens on yield (a measure of the carbon fixed into complex molecules such as starch, fats, and structural components in the plants).

In a table, present the weights of each plant part and also express these as a percentage of the control (complete nutrient solution). Data should also be presented from the entire class and analyzed using appropriate statistical tests. This average data could be presented in appropriate visual form, e.g., as box plots.

Acknowledgement: The makeup of the nutrient stocks was adapted from recipes on the University of Guelph Department of Botany webpage “Hydroponics in the Classroom” at http://www.cpes.uoguelph.ca/STAO/hydrop.html.
LAB 5: Phytochrome effects on the germination of lettuce seeds

25-Jan

1. Purpose:

To demonstrate the reversible light requirements of Grand Rapids lettuce seeds.

2. Introduction:

Many investigators have demonstrated that red light is the most effective color for breaking dormancy of light-requiring seeds. Evidence that the phytochrome system is involved in such cases of promotive germination comes from observations that (1) the red light effects resulting from absorption by Pr can be overcome by far-red light subsequently absorbed by Pfr, and (2) relatively small amounts of red or far-red energy are needed to effect germination. We do not yet understand how Pfr promotes seed germination. We do know, however, that the seeds must imbibe a certain amount of water before light is effective. This may be due to the requirement for moisture before Pr can be converted to Pfr. The photoreversibility of germination in Grand Rapids lettuce seeds and the time of light sensitivity will be studied in this experiment.

3. Materials: (two dishes/treatment)

- 50 Grand Rapids lettuce seeds per petri dish
- 5 cm diameter glass petri dishes with two layers of filter paper/treatment (11 treatments)
- automatic pipettor with tips
- marking pen
- aluminum foil
- dark room with green safelight and red/far-red lights
- deionized water
- thermometer

4. Procedures:

A. Pre-conditioning:

Prepare 14 dishes with 50 seeds and 4 ml of water in each (8 others have been prepared for you one day earlier - treatments 2, 4, 6 and 8). Pre-condition the seeds under far-red light for one hour (make sure the temperature does not increase above 25C or the seeds will become thermodormant).
B. Treatments:

After one hour of pre-treatment conditioning, start the following treatments, giving all red and all far-red light simultaneously (i.e. plant!):

1) White light (bench top) control
2) White light (bench top) control (after 24 hours imbibition in the dark)
3) Dark control
4) Dark control (24 hours of imbibition in the dark)
5) Red light (1 minute)
6) Red light (1 minute, after 24 hours imbibition in the dark)
7) Far-red light (5 minutes)
8) Far-red light (5 minutes, after 24 hours imbibition in the dark)
9) Red (1 minute) + Far-red (5 minutes)
10) Red (1) + Far-Red(5) + Red (1)

After light treatments have been given, wrap all dishes except No.1 and 2 in foil and place in a dark box for 48 hours (i.e. make sure the seeds don't get exposed to light other than that shown above!!) Monitor the temperature so that bench top and dark box are similar. After 48 hours, determine % germination for each treatment.

5. Report

Count % germination and prepare a bar graph. Report averages +/- SDs.

If the results are not as you expected, why might that be?

6. References


LAB 6: The effect of plant growth regulators on the elongation of dwarf pea seedlings

WEEK 1: 08-Mar
WEEK 2: 15-Mar

1. Purpose:

To study effects of plant growth regulators on the stem elongation in dwarf peas.

2. Introduction:

In lecture, you will have learned that several of the plant growth regulators have significant effects on the growth of various plant organs. In this lab you will survey the effects of three compounds on the elongation growth of dwarf pea stems.

3. Materials:

- Five pots, each with four 1-week-old etiolated pea plants (Progress #9)
- Ruler
- Paintbrushes
- 100 mL distilled water (with Tween 20 – 6 drops per litre)
- 100 mL of 0.1 mM gibberellic acid, made in Tween 20 solution (6 drops per litre)
- 100 mL of 0.1 mM indoleacetic acid, made in Tween 20 solution (6 drops per litre)
- 100 mL of 0.1 mM kinetin made in Tween 20 solution (6 drops per litre)

4. Procedures:

Measure and record the height of each plant in all four pots. Paint all plants in one pot with water only (control) and the treatment plants with each appropriate solution (four plants per pot per chemical). Make sure there is no transfer of hormones between pots, i.e. that there is no contact among plants in different pots. Grow the plants in a greenhouse for one week, measure again and prepare a bar graph showing your results.

5. Report:

In your report, concentrate on the theoretical mode of action of each growth regulator (on elongation only!) and relate this to your own observations. In one sentence, why are some growth regulators effective in only some tissues of some species but not others?

6. References:

1. BROUGHTON W J and A J McCOMB 1971 Changes in th pattern of enzyme development

2. BURG SP and EA BURG 1966 The interaction between auxin and ethylene and its role in plant growth. proceedings of the National Academy of Sciences 55: 262-269.


For additional references to be used in this and the following plant growth regulator labs, search the journal Plant Physiology (www.plantphysiol.org) using the keywords: auxins, gibberellins, cytokinins. Concentrate on papers dealing with: growth (wall extension), chlorophyll synthesis (greening), alpha-amylase synthesis and seed germination (particularly barley and peas).
**LAB 7: The water potential of potato tubers**

15-Mar

1. **Purpose:**

To study one aspect of plant water relations. Namely, to compare the water potentials of young, old, and freeze-damaged potato tubers.

2. **Introduction:**

This experiment demonstrates some of the principles of plant water relations without the need for elaborate equipment. The principle of this method is related to the observation that tissue placed in an isotonic solution neither gains nor loses water. (See your textbook for a pictorial depiction of this experiment). The changes in water content of tissue placed in solutions of varying water potentials can be determined by measuring changes in fresh weight or volume of the tissue. The measured water potential values tend to be more negative than those measured by other methods. This seems to be due to the uptake of additional solution into the apoplastic space (intercellular space and cell walls). This uptake is of course independent of the osmotic potential of the protoplasts, but is partially a measure of the matrix potential of the cell walls.

3. **Materials:**

- Potato tubers: **young; old and sprouting;** damaged by freezing at -50°C
- 2 L of 1 M sucrose (household sugar) at room temperature (500 ml/group)
- 4 x 250 mL beaker (three with lids, and lined with moist paper towels)
- 100 mL graduated cylinder
- 18 petri dishes plus lids (alternatively can use small beakers)
- 3 or 4 - mm diameter cork borer
- Marking pen and ruler
- Razor blade
- Sensitive balance
- Blotting paper
- 10 ml pipettes and bulbs

4. **Procedures:**

Obtain 500 mL of a 1 M sucrose stock solution. Dilute proper aliquots of this into 200 ml beakers to make 100 mL of 0.15, 0.20, 0.25, 0.30, 0.35 and 0.50 M sucrose (make sure you know how to prepare these before you come to the lab). Mix well and divide the solutions into three series of Petri dishes. Label the bottoms of the dishes to avoid confusing the lids. Cut out 18 or more cylinders using the cork borer from each type of potato. Store the pieces in a large beaker with moist paper towels covered with a lid, to prevent excessive evaporation. Cut
each piece to exactly 20 mm length. Randomly select three pieces per treatment, blot off excess moisture, weigh to the nearest 0.01 gram and place them into appropriate solution. Incubate the pieces for at least three hours in order to establish osmotic equilibrium. Remove the pieces, blot off excess moisture, weigh the pieces again. Tabulate, for your own use, the initial weight, final weight and % change in weight.

5. Report:

Do not present tables, instead, plot % change for each type of tuber against sucrose concentration. Intercept of the 0% change line, extrapolated to the appropriate sucrose concentration represents a close approximation of the water potential of the young tuber tissue. Discuss why this is or is not the case, the potential sources of error, and the osmotic behavior of the old or damaged tissue.

6. References:

