WHAT IS PLANT TISSUE CULTURE?

Plant tissue culture involves the growth of plant cells, tissues or segments for purposes such as generating or cloning large amounts of new cells, tissues or plants; to study development; to provide mechanisms of genetic engineering; or to produce valuable chemicals found in plant cells. Cloning is not an alien concept to those people familiar with plants. It is a process of generating exact and multiple copies of a parent plant, Cloning can be done by making cuttings, grafts, or even naturally, such as the "babies" or propagules sent off by spider plants. Plant tissue culture uses much smaller pieces of tissue in this cloning process. Of course, there are so many possibilities in this field that plant tissue culturists can do more than simply clone plants. Plant tissue culturists perform a number of different types of experiments to place new plants into culture or to develop new methods of working with plant cultures.

The plant tissue culturist should have a good idea at the outset as to the purpose of the experiments. There are a number of different types of *in vitro* tissue culture approaches. For tissue culture, the term *in vitro* refers to growing pieces of plants separate from the entire organism. We will explore some of these in our laboratory. Before we begin our experiments, it is helpful to describe some of the more important types of tissue culture.

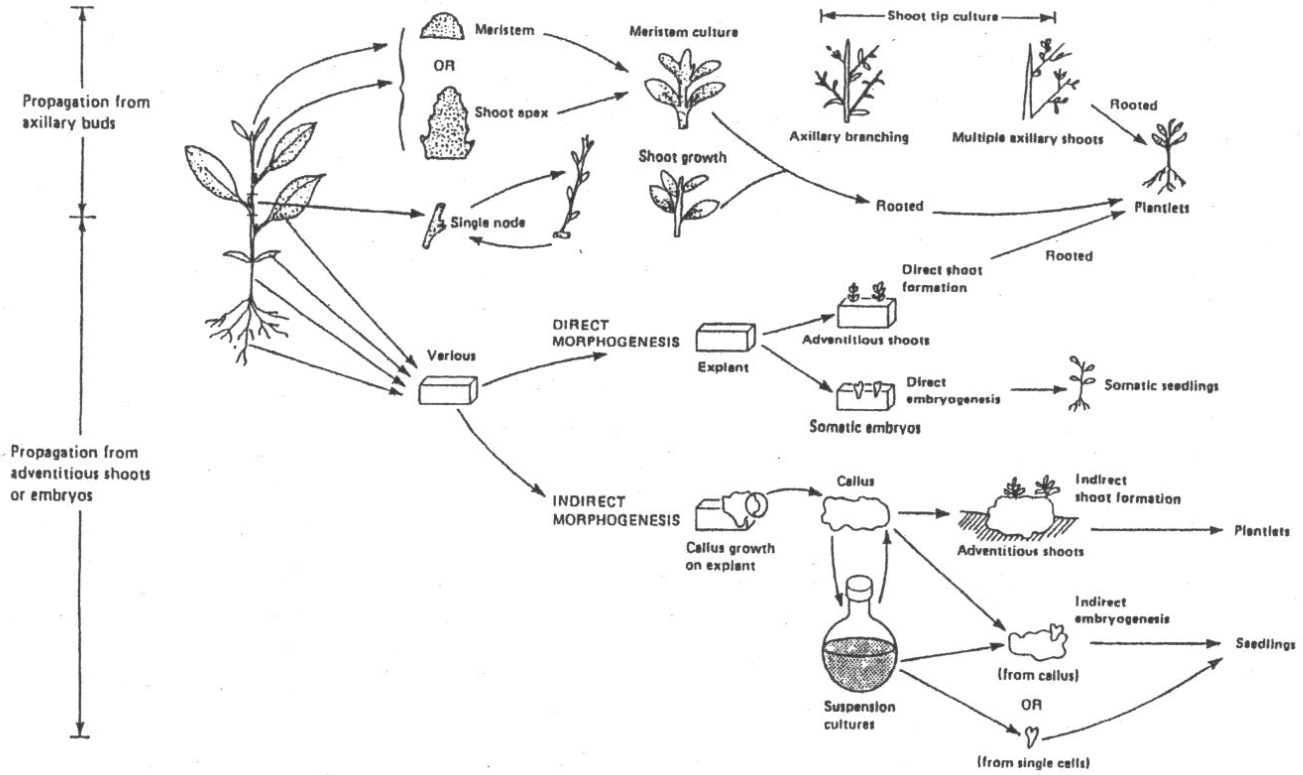
MICROPROPAGATION: Micropropagation uses small pieces of tissues such as axillary buds, tubers or rhizomes for rapid cloning or generation of new plants. Think of a bud on a plant. The bud contains the meristematic area. Under *in vivo* (on the plant) conditions, growth is carefully controlled by different plant growth regulators (which ones?). Under these conditions, a bud continues to grow as part of a whole system. The tissue culturist excises the bud and alters the plant growth regulators. Growth then changes and multiple shoots will arise from a single bud. Each of these shoots have buds, which in turn can be cultured. The culturist can repeat this process over and over again to produce hugh numbers of buds, each capable of producing a whole plant. Similar multiplication steps can be performed with rhizomes, nodes, tubers or other plant part. Micropropagation is the type of tissue culture used in the commercial horticulturist industry.

ORGANOGENESIS: Organogenesis refers to the development of plant organs such as leaves, roots or shoots from undifferentiated callus. A callus is a mass of "unorganized" plant cells. Callus can occur as a result of a plant wound. In plant tissue culture, it usually arises from pieces of tissue which the culturist places on a medium containing certain plant growth regulators. When the culturist moves the callus onto a new medium with different plant growth regulators, the cells in the callus are induced to organize and form new plant organs.

EMBRYOGENESIS: Plant embryos can arise from the manipulations of certain calluses. These embryos arise from undifferentiated cells in the callus. They reform into embryos. The term indirect embryogenesis is used when the embryos arise from a callus. If the embryos form from developed plant tissue, the term direct embryogenesis is used. Generally, embryogenesis is defined as a embryo arising from a single cell.

EMBRYO/OVULE CULTURE: In some plants, embryos or ovules have insufficient nutrient reserves to develop and germinate. Orchids are excellent examples. The seeds of orchids must be placed on a nutrient medium to allow full germination. This medium contains important salts, vitamins, sugar and plant growth regulators. The latter may improve germination or overcome any germination blockers.

PROTOPLAST CULTURE AND USES: When a plant cell is stripped of its wall, it is bound only by its plasmalemma. This cell is called a protoplast. Such cells may be fused with other cells to create entirely new species. This process is called protoplast fusion. The new fused protoplasts are cultured to reform new cell walls. The complete plant cells are then cultured back into new plants. Protoplasts can also be excellent choices for the injection of foreign DNA.



The principal methods of micropropagation
Shoots and/or plantlets do not always originate in culture by a single method

WHY PLANT TISSUE CULTURE?

There are a lot of different kinds of experiments we could do in a Plant Physiology lab, and we will do some traditional physiological experiments. Even before the course started, you probably imagined projects on photosynthesis, flowering or growth. After skimming your textbook, you will become aware of major research areas in plant physiology such as translocation, nutrients, photoperiodicity and so on. So why will our laboratory exercises focus on plant tissue culture?

First, let us consider some of the practical reasons. We grow plants for their many products - food, fiber, medicinal products, aesthetic value and energy. Directly or indirectly, plants and their products account for 35% of our nation's gross domestic product. This is a measure of the value of goods produced in the Unites States. Growing plants is obviously important!

The significance of plants to our economy leads to efforts to genetically improve plants, to provide new types of plants for different uses, and to develop new products from plants. While classical plant breeding and propagation is still an active component of plant improvement, more businesses and scientists are looking to the growing fields of biotechnology to provide new methods of plant improvement.

Scientists are now able to insert genes from other plants, bacteria or even animals into their "target plants." Almost without exception, plant biotechnologists use some type of plant tissue culture in this process. Plant biotechnologists generally start with single cells or a very small amount of tissue as their basis for recombinant DNA technology or some other form of genetic selection. Once these cells are "genetically engineered", scientists have to culture these cells and regenerate whole plants back from those cells. Plant tissue culture is therefore an important component of plant biotechnology.

Plant tissue culture technology is already commercially viable. Horticulture businesses propagate many of our favorite household plants such as peace lilies, boston ferns, and african violets through the tissue culture process we call micropropagation. Other scientists are looking at using this technology for producing indigenous plants used in environmental restoration.

When students work in plant tissue culture, they not only learn science, but acquire important laboratory skills as well. These are the kinds of skills that lead to better careers through government, teaching, business, or acceptance into good graduate schools. Plant tissue culture is an employable skill and it is worth mentioning on any resume.

With all these practical reasons aside, there are some good intellectual reasons. Plant tissue culture integrates almost all the various aspects of plant physiology. The responses of plants in tissue culture vary as you alter the nutrients, plant growth regulators, photoperiod, temperature and even the type of tissue used for the cultures. Plant tissue culture is an excellent way for students to learn how their class lectures relate to some of the most important processes in plants: growth and development.

LABORATORY TECHNIQUES FOR PLANT TISSUE CULTURE

Proper tissue culture procedures require aseptic techniques. Those students who have taken microbiology are already familiar with these techniques. The culturist is the biggest factor in maintaining axenic cultures. Following good, sterile technique, the laminar flow hood represents a major breakthrough for tissue culture. These hoods filter the incoming air of all particles greater than 0.2 μm in size. The hood directs a gentle (even unnoticeable) stream of sterile air out of the enclosure towards the culturist. This prevents air-borne particles from blowing into the work area. As the cultures are manipulated in the space of the hood, the airstream creates a sterile zone and the flow prevents airborne microbes from landing in the culture vessels or on the plants.

In practice, the culturist must perform all media and tissue preparation operations using sterile techniques so that foreign microbes are not introduced into the axenic cultures. With the high sucrose and nutrient content of the media, heterotrophic microbes would have a wonderful environment in which to grow, multiply and over-run the cultured plants.

Good techniques call for:

1 . Wearing clean clothing and keeping long hair away from the cultures.
2. Cleaning the laminar flow hoods prior to and after use.
3. Keeping fingers or other body parts downstream and away from the cultures. This requires that the vessels be manipulated carefully and in the sterile air flow.
4. Keeping the tools, i.e. scalpels, forceps, etc clean and sterile through frequent dips in 70% ethanol followed by heat treatments.

These techniques will become clearer when your instructor demonstrates them to you.

Cleanliness, good sterile technique, and attention to keeping your airway clear is of paramount importance for success in any plant tissue culture!

MEDIA

Since plant tissue culture involves the in vitro culture of plants or cells in small containers, careful attention must be paid to the plants' nutritional needs. In addition, small pieces of plants may not be able to manufacture all their own food through autotrophic means (what is autotrophic?). The culturist usually needs to supply some other form of energy rich compound, such as sucrose. Also, the medium should contain the kinds of plant growth regulators which the culturist hopes will produce the desired forms of growth. Such growth might be rapid shoot multiplication, callus formation, organogenesis or embryogenesis.

Plant tissue culture media are made up of many components. These include inorganic salts, sucrose or some other carbon source, plant growth regulators and vitamins which the plants are often unable to make for themselves. The major types of media are shown in Table I.

Another important feature of the different media is the pH. The pH affects both the growth of plants and the activity of the plant growth regulators. A pH of 5.7 is used for most plant tissue culture applications.

The culturist prepares media in several ways. The two most common approaches are to prepare stock solutions or buy the salts in premixed bottles (major suppliers include Sigma, Gibco or Carolina Biological Supplies). Methods for making up stock solutions of some common plant growth regulators are included in Table 2.

The first step of the process is to measure out about 70-80% of the desired volume of distilled water needed. In other words, if the culturist needs 1 L of a medium, 700-800 mls of distilled water are poured into an appropriate flask. (a volume closer to 700 ml is used if liquid stock solutions provide the nutrients, while as much as 850 ml can be used if premixed salts provide the nutrients). The salts are dissolved in the water. The culturist then adds sucrose, usually around 1-to-3% (w: v for 1% = 1 g for every 100 ml medium). Both vitamins and plant growth regulators are added from stock solutions. The different types of plant growth regulators and methods for making stock solutions are shown in Table 2. After insuring that everything is dissolved, the culturist adjusts the pH (usually 5.7). The medium is then poured back into a graduated cylinder where it can be adjusted to the final volume.

The media may be supplied to the plants in a liquid form or it may be solidified with agar. Agar is a polysaccharide from marine red algae which produces a gel when dissolved in water and allowed to cool. In plant tissue culture, the agar content of a solid medium is around 0.8% w: v (how many g of agar per 1 L?). In order to dissolve the agar, the culturist heats the medium to boiling with the agar in it. After the medium becomes translucent, the agar is dissolved.

The medium is then dispensed into the containers. The individual containers with their lids
are then autoclaved to sterilize the medium (121 ^{o}C, 15 psi, 20 minutes). In some instances, plant growth regulators may not be autoclavable. In these cases, the plant growth regulators are filter sterilized (through a 0.2 μm filter) and added to the medium after it is autoclaved. In such cases, the containers may be sterilized without the medium. When the fully complete medium is finished, it is then poured into the containers.

TABLE I
FORMULATIONS OF SELECTED PLANT TISSUE CULTURE MEDIA

Compound	Murashige Skoog	Woody Plant Medium	Anderson	White's	Gamborg B5	Heller
	concentration (mg/liter)					
NH_4NO_3	1650	400	400			
KNO_3	1900		480	80	2500	
$Ca(NO_3)_2 \cdot 4H_20$		556		300		
$NaNO_3$						600
$(NH_4)_2SO_4$					134	
$MgSO_4 \cdot 7H_2O$	370		370	720	250	
$NaSO_4$				200		
$CaCl_2 \cdot 2H_2O$	440	96	440		150	75
KH2PO4	170	170	380			125
$NaH_2PO_4 \cdot H_2$				16.5	150	
KCI				65		750
K_2SO_4		990				
$MgSO_4 \cdot 7H_20$		370				250
$FeSO_4 \cdot 7H_20$	27.8	27.8	55.7		27.5	
Na_2EDTA	37.3	37.3	74.5		37.3	
$FeCL_3 \cdot 6H_20$						1
$Fe_2(SO_4)_3$				2.5		
$MnSO_4 \cdot H_20$	16.8	22.3	16.9		10	0.01
$ZnSO_4 \cdot 7H_20$	8.6	8.6	8.6	3	2.0	1
H_3BO_3	6.2	6.2	6.2	1.5	3.0	1
KI	0.83			0.75	0.75	0.01
$Na_2MoO_4 \cdot 2H_20$	0.25	0.25	0.25		0.25	
$CuSO_4\ 4 \cdot 5H_20$	0.025	0.25	0.025		0.025	0.03
$CoCl \cdot 6H_20$	0.025		0.025		0.025	
$NiCl \cdot 6H_20$						0.03
$AlCL_3$						0.03
myo-inositol	100	100	100		100	
nicotinic acid	0.5	0.5		0.5	1.0	
pyridoxine HCl	0.5	0.5		0.1	1.0	
thiamine HC1	0.1	1.0		0.1	10.0	1.0
glycine	2.0	2.0		3.0		
Ca D-pantothenic acid				1.0		

TABLE 2.
PLANT GROWTH REGULATORS FOR TISSUE CULTURE

General Formulations

Most plant tissue culture labs make up their plant growth regulators as stock solutions. A stock solution is a concentrated solution of a desired chemical. When the chemical is needed, a small amount of stock solution is added to a medium. This avoids having to weigh out frequent and small amounts of plant growth regulators.

Most stock solutions for tissue culture are made so that 10 mg of chemical can be found per 1 ml of stock solution. To make a stock solution, 100 mg (0. 1 g) of plant growth regulator is weighed out and dissolved in some form of solvent. (many plant growth regulators dissolve poorly in water). Enough water is added to make a final stock solution of 10 mls. For example, 100 mg of IAA is dissolved in 2 ml of 0.5 N NaOH. After the IAA is dissolved, 8 mls of water are added. The stock solution is stored in the refrigerator until needed. Storage times vary, but they should never be longer than a month.

Plant Growth Regulator (Abbreviation)- Solvent

Auxins:
Indole Acetic Acid (IAA)- 0.5 N NaOH
Indole Butyric Acid (IBA)- 0.5 N NaOH
Napthateneacetic Acid (NAA)- 0.5 N NaOH
2,4 Dichlorophenoxyacetic acid (2,4D)- 95% ethanol

Cytokinins:
kinetin (K)- 0.5 N HCl
zeatin (Z)- hot, not boiling water (Do not autoclave, filter sterilize)
benzylaminopurine (BA or BAP)- 0.5 N HCl
2-isopentyladenine (2iP)- 0.5 N HCl

Others:
Abscisic Acid (ABA)- 0.5 N NaOH
Gibberelic Acid (GA) - hot, not boiling water (Do not autoclave, filter sterilize)

Plant Growth Regulator Effects

Auxins
- cell elongation
- cell wall relaxation
- increased RNA and protein synthesis
- direction of translocation
- prevents abscission
- enhanced ethylene production
- organ formation (with cytokinins)

Cytokinins
- cell division
- cell enlargement
- organ formation (with auxins)
- overcomes dormancy
- lateral bud development
- retards senescence
- mobilization of nutrients

Gibberellins
- cell elongation at apex
- Cell division
- enzyme induction
- flowering (long day)
- overcomes dormancy (anatagonist to ABA)
- inhibits organ formation

Abscisic Acid
- dormancy
- gibberellin anatagonist
- flowering (short day)
- stomate closure during water stress

Ethylene
- enhanced production with high auxin concentrations
- epinasty
- geotrophism
- fruit ripening
- flowering of mangos and bromeliads
- senscence
- abscission

MEDIA PREPARATION LAB

Rose Shoot Multiplication and Rooting

Plant culture media is composed of many ingredients. Key ingredients are: an alternative carbon source such as sucrose, plant growth regulators, inorganic salts and vitamins - all of which are dissolved in DI H20. The pH is then adjusted to a value between 5.4-5.7. Agar is another constituent that is added to make a solid media, added after the other ingredients have been added and the pH has been adjusted. Agar is the ingredient responsible for the "gel-like" quality of solid media. Many laboratories use pre-made nutrient solutions to save time. However, solutions can be prepared in the lab. Once the medium is complete, it must first be sterilized before it can be used. The autoclave is what sterilizes plant tissue media through increased temperature and pressure. In general, making plant media is very similar to cooking!

In lab, each group will make up 100 ml each of Rose shoot initiation/multiplication and rooting medium. These media are composed of the following:

Rose multiplication media salts (4.41 g/L)
0.3 mg/L IAA- in the multiplication medium
2.0 mg/L BA - in the multiplication medium
Rose rooting media salts (1.18 g/L) - contains 0.01 mg/L NAA

Both media also contain:
3% sucrose, pH 5.7, and 0.8% agar

1) Fill each of your group's beakers with 80 ml of DI H20. Add a stir bar and begin to stir at medium speed.

2) Calculate the quantities of salts, sucrose and agar you need. Add the salts, then sucrose, to your medium. **Do not add the agar at this time!**

3) Remove the beaker from the stir plate and pour the medium into a graduated cylinder. Bring the volume up to 100 ml. After this is done, pour the medium back into the beaker and stir.

4) Determine the pH of your group's medium. Adjust it to 5.7 using the 0.1N NaOH and HCI provided. Add a drop or two at a time and be patient.

5) Add the calculated amount of agar and heat the media until it becomes clear (80 C) .

6) After the agar has dissolved, pour about 25 ml in each of 4 culture tubes and cap with vented closures. Use autoclave gloves!

7) Autoclave.

8) Once the autoclave cycle is complete, all media are sterile! After media has cooled, transfer your group's tubes to the laminar flow hood.

pH Meter Instructions

1. Turn white ring at top of the electrode to open

2. Carefully remove cover from the electrode tip and rinse with dH_2O

3. CAREFULLY place the electrode into the media - make sure the stir bar will not hit the electrode tip!!!

4. Turn the left-mots dial on the pH meter fro Standby to pH

5. Wait until the pH reading stabilizes and record pH

6. Add acid or base solution until the correct pH is reached

7. Turn the dial back to standby

8. Remove electrode from media, turn the white ring back to the closed position, rinse the tip with dH_2O, and gently replace the cap on the electrode tip.

MICROPROPAGATION WITH MINIATURE ROSES- MULTIPLE SHOOT PRODUCTION FROM AXILLARY BUDS

Objective: To compare explant type and the effects of media with and without cytokinins on the production of shoots from axillary buds.

One of the most often used methods of micropropagation is the use of axillary buds. Students should review some of the suggested roles that active meristematic areas play on other meristems. In essence, apical dominance inhibits the formation of lateral buds. In axillary bud cultures, shoot segments with several axillary buds are placed on a medium high in cytokinin. The cytokinin overcomes inhibition of the axillary buds by auxin from the apical meristems. Multiple shoots can arise from a single bud, giving a highly branched shoot in culture. Of course, each of these shoots can be subcultured to produce more new shoots resulting in an efficient propagative system for the production of new plants.

Stage II. Shoot Multiplication

Students will be provided established miniature rose plants. Shoots should be removed from these cultures. It is not necessary to remove each axillary bud, rather each shoot can be cut into 2 sections from the top and bottom of the shoot. Each section will be transplanted onto rose medium. It is important to keep node sections moist at all times!

Two media will be used. One will contain the auxin IAA and the cytokinin, BA and the other will contain the auxin NAA, but no cytokinin. The basic rose medium is MS salts, woody plant vitamins, 3% sucrose, 0.03 mg/l NAA, and 0.8% agar, at a pH of 5.7. The experimental media will have 0.3 mg/L IAA and 2.0 mg/l BA. Students should also compare the effectiveness of shoot sections removed from the top and base of the excised shoots in producing new shoots and for each explant type compare shoot production for media with and without additional cytokinins..

Stage III. Rooting (done after 8-10 weeks in culture).

As miniature rose cultures grow and age, they produce roots even within the multiplication stage. For roses, this eliminates the costly requirement for a separate rooting stage. In some types of plants cultured through axillary buds, it may be necessary to have a stage III rooting trial if there are too few roots per shoot or too many roots per shoot.

To prepare rose shoots for the greenhouse, students should carefully remove the shoots from the medium. The agar should be washed off under running water. The roses are potted in a mixture of perlite and potting soil. The plants will be hardened off in the misting system of the greenhouse.

FLOW CHART FOR ROSE STEM STERILIZATION

TAKE 1-3 CM CUTTING FROM ACTIVELY GROWING SHOOT TIP

⇓

REMOVE ALL LEAVES
(EXCEPT THE SMALLEST TERMINAL LEAVES, IF USING STEM TIP)

⇓

WASH SHOOT SECTIONS IN WATER WITH A FEW DROPS OF DETERGENT
(USE PLASTIC PETRI PLATE)

⇓

RINSE EXPLANTS IN DI WATER

⇓

TRIM SHOOT TO EXPLANT SIZE W/STERILE SCALPEL IN HOOD
(MAKE SURE EXPLANT INCLUDES NODE W/LATERAL BUDS)
(TRIM EXPLANT IN PETRI PLATE)

⇓

SURFACE DISENFEST IN 70% EtOH FOR 30 SEC

⇓

RINSE EXPLANTS IN DI WATER

⇓

SURFACE DISINFEST IN 20% CHLOROX IN BEAKER FOR 20 MIN
(SWIRL BEAKER TO ENSURE CONTACT)

⇓

RINSE EXPLANTS IN 3 CHANGES OF STERILE WATER
(IN SCINTILLATION VIALS)

⇓

ASCEPTICALLY TRANSFER EXPLANTS TO CULTURE TUBES
WITH ROSE SHOOT MULTIPLICATION MEDIUM
(BE CAREFUL TO AVOID CONTACT WITH TUBE TOP EDGE)
(LAY EXPLANT ON SIDE SO THAT BOTH CUT ENDS CONTACT AGAR)

⇓

LABEL TUBE W/DATE, *Rosa* AND INITIALS AND PLACE IN CULTURE RACK

⇓

YOU WILL MONITOR THESE EXPLANTS EACH WEEK FOR CONTAMINATION AND
FOR SHOOT PRODUCTION .

MULTIPLE SHOOT PRODUCTION OF MINIATURE ROSES FROM AXILLARY BUDS							
Weeks in Culture	Media	# clean cultures	# contaminated	# cultures with new shoots	Mean # shoots per explant	# cultures with roots	Mean # roots per explant

NUTRIENT DEFICIENCIES IN PLANTS

In order for plants to grow and reproduce normally, they need an adequate supply of essential elements. When one or more of these elements is absent, plants display deficiency symptoms. Generally, these symptoms include stunted growth of roots, stems or leaves and chlorosis or necrosis of various organs.

The characteristic symptom displayed by a plant in response to the absence of an element can give us insight into what the functional role of the element may be. For example, if leaves fail to develop a green pigment, one can conclude that the plant may be missing an element that plays a key role in the synthesis of chlorophyll. Other functions that elements play a significant role in are: protein synthesis, photosynthesis, respiration and translocation.

Diagnosing which elements are absent becomes increasingly difficult when dealing with multiple deficiencies. So, for the purposes of our study, we will observe plants deficient in only a single element Seven different nutrient solutions were prepared prior to lab using the attached table. In this lab, we will examine deficiency symptoms for the following elements: nitrogen (N), potassium (K), iron (Fe), calcium (Ca), phosphorous (P), and magnesium (Mg).

PROCEDURE

In order to examine the 6 nutrient deficiencies, we will use the plant *Lycopersicon esculentum* (tomato). Each group will be assigned a nutrient solution missing only one nutrient. Within each group, 3 plants will be exposed to the solution. The experiment will run for 2 months. In order to compare each group's plants to healthy, "normal" plants, one control group of 3 plants, provided with all of the essential nutrients, will also be grown.

In total, we will expose 21 plants to 7 different nutrient solutions.

1. Into each of your 3 GA-7 vessels pour 250 ml of your group's assigned solution. Put lids on vessels. Do not overfill as it will rot your stems!
2. Remove plants from peat pots and rinse them off well, especially roots, with tap water. At this time, take note of your plants' overall appearance (color, morphology, etc.). Also measure and record the height of each of your group's plants and mark the level of the solution on the side of the flask.
3. Insert plant stem into hole in lid, stem should be submerged in solution. Secure the position of the plant by putting filter floss in the hole around the stem. The extra hole is for aeration. Do not get the floss wet - it will rot your stems!
4. Cover each vessel with aluminum foil to inhibit algal growth. Be sure to leave top uncovered.
5. Take plants to the greenhouse. At weekly intervals check your plants and maintain your solution level by adding distilled water. Measure plant height and # of leaves. Also, record observations on leaf color, shape, necrosis, chlorosis, etc.
6. At the end of the experiment, make final observations on the appearance of your plants and measure the plant height leaf #. In the lab, separate roots from shoot/leaves and place on weigh pans in drying oven (@60 C) for at least 48h and weigh to determine final biomass.

NUTRIENT SOLUTION ADDITIONS (ml/l) FOR NUTRIENT DEFICIENCY LAB

		NUTRIENT DEFICIENCY*						
STOCK SOLUTIONS	Molarity	NONE	N	P	K	Ca	Mg	Fe
A - Ammonium Phosphate	0.20	5	–	–	5	5	–	5
B - Ammonium Nitrate	0.50	–	–	1	6	8	6	–
C - Calcium Nitrate	1.15	5	–	5	5	–	5	5
D - Calcium Chloride	0.26	5	21	5	5	–	5	5
G - Magnesium Sulfate	0.40	5	5	5	5	5	--	5
H - Potassium Phosphate	0.20	–	5	–	–	–	5	--
I - Potassium Nitrate	1.20	5	–	5	–	5	1	5
J - Potassium Sulfate	0.50	–	5	–	–	–	4	--
L - Microelements		2	2	2	2	2	2	2
Boric Acid	1.20							
Copper Chloride	0.012							
Manganese Chloride	0.23							
Zinc Chloride	0.044							
Molybdic Acid	0.006							
M - Fe EDTA		2	2	2	2	2	2	--

*All numbers represent ml of stock solution to be used per liter of test solution

Tomato Plant Height (cm)								
Defficiency	Rep	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
None	1							
	2							
	3							
Nitrogen (N)	1							
	2							
	3							
Phosphorus (P)	1							
	2							
	3							
Potassium (K)	1							
	2							
	3							
Iron (Fe)	1							
	2							
	3							

Tomato Plant - Leaf Number								
Defficiency	Rep	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
None	1							
	2							
	3							
Nitrogen (N)	1							
	2							
	3							
Phosphorus (P)	1							
	2							
	3							
Potassium (K)	1							
	2							
	3							
Iron (Fe)	1							
	2							
	3							

Tomato Plant - Observations								
Defficiency	Rep	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
None	1							
	2							
	3							
Nitrogen (N)	1							
	2							
	3							
Phosphorus (P)	1							
	2							
	3							
Potassium (K)	1							
	2							
	3							
Iron (Fe)	1							
	2							
	3							

PLANT PHYSIOLOGY DRY WEIGHT DATA						
DATE	**TREATMENT**	**TISSUE**	**PAN#**	**PAN+TIS wt**	**PAN wt.**	**TISSUE wt**

ORGANOGENESIS OF TOBACCO, *NICOTINA TABACUM*

Tobacco is a major model system in plant biotechnology, much like *E. coli* is for microbiology. Tobacco demonstrates organogenesis very easily. This is also a good exercise to investigate the effects of different ranges in auxin and cytokinin concentrations on plant development.

Objective: To demonstrate the process of organogensis from callus cultures and the effects of plant growth regulators on organogenesis. Organogenesis is the process where new plant organs differentiate from a seemly undifferentiated mass of tissue, the callus. In fact, a look at calli will reveal that they are themselves composed of multiple cell types.

Callus Induction

Organogenesis refers to the regeneration of plant organs such as roots and shoots from apparently undifferentiated cell masses. In order to first create a mass of undifferentiated cells called callus, it is necessary to initiate callus development Portions of stem, root, embryo or sometimes leaf tissue can be used to initiate callus. After such calli are formed, they may be broken up and grown as isolated cells which can be genetically engineered. The isolated cells are allowed to reform callus, and organogenesis used to initiate development of the whole plant, which hopefully is genetically transformed.

Each group will be given a tobacco plant. The top 3 cm should be removed and the leaves cut off. The segment should be washed in water. The segments should be surface sterilized with a 30 sec rinse in 70% EtOH followed by a 20 min soak in 20% Chlorox and two sterile water rinses. After surface sterilization, the sections should be placed on a sterile surface. Cross sections of approximately 2-3 mm in thickness should be cut, after discarding any end sections. The epidermis should also be cut away. These sections should be laid flat on callus inducing medium in the petri dishes, with 2-3 sections per petri dish. The edges of the closed petri dishes should be sealed with Parafilm.

The callus inducing medium contains MS salts, MS vitamins, 3 mg of IAA (auxin)/l, 0.2 mg of kinetin (cytokinin)/l, 3% sucrose, and 0.8% agar, at a pH of 5.6.
After callus is induced, we will use these cultures for experiments in organogenesis.

Organogenesis 4-6 weeks after induction lab

You began the process of callus induction by placing tobacco pith on a callus induction media. You will use your calli or those supplied by your instructor to examine the effects of different auxin:cytokinin ratios on shoot and root proliferation from callus.

The three media we will test are all based on the same formulation as the callus induction medium, but the ratios and concentrations of auxin (IAA) and cytokinin (kinetin) have been varied:

1.) Shoot induction media: 0.03 mg IAA/l: 1.0 mg kinetin/l (1:3 auxin:cytokinin)
2.) Root induction media: 3.0 mg IAA/l: 0.02 mg kinetin/l (150:1 auxin:cytokinin)
3.) Callus media 2.0 mg IAA/l: 0.2 mg kinetin/l (10:1 auxin:cytokinin)

Students should use good sterile technique. Remove the calli from the petri plates, and place one callus on each of the three media. Recap the tubes and seal with Parafilm.

Students should note the changes that occur in the tobacco calli over the periods of the next several weeks. In particular, note which kinds of cells seem to give rise to the new organs.

Water Potential

Water potential is the sum of solute, pressure and gravity potentials. In an open system the effects of hydrostatic pressure and gravity are negligible leaving the solute or osmotic potential as the greatest contributing term. Solute potential is a function of dissolved solutes in water and is a colligative property meaning it all the particles dissolved in water act in an additive manner. When the osmotic potential is equal for a tissue and the surrounding water there is no net movement of water. If the tissue has a lower osmotic potential then water moves in and vice versa. We will use this principle to determine the osmotic potential of a tissue using solutions with known osmotic potentials.

Experimental Procedure

1. Mannitol solutions of 0 m, 0.1 m, 0.2 m, 0.3 m , 0.4 m, and 0.5 m (m=molality) are provided.
2. Pipette 10 ml of each of the above concentrations into separate wells in the 6-well multiwell plate. Mark on the lid, above each well, the concentration it contains. Set aside.
3. Using a size 4 cork borer, obtain bored samples of potato. Cut all pieces down to approximately 2.5 cm (1 in) in length. You will need five 2.5 cm segments.
4. Make a chart labeled with the 5 concentrations and assign a potato segment to each concentration. Record the weights of the potato segments next to the concentrations on a sheet of paper.

c	t_0 Wt (g)	Wt@0.75h(g)	Wt@1.5h(g)	Δ%Wt
0	0.67			
0.1	0.55			
0.2	0.66			

5. After all of the weights have been recorded, put each potato segment into the well marked with the concentration that corresponds to the one on your sheet.
6. At 45 min intervals for the next 1 1/2 hours, reweigh the potato cores. Be sure to pat them dry with a paper towel to remove excess water prior to weighing.
7. Calculate % weight change Δ%Wt = ((final wt - start wt)/start wt)*100, regress % wt change vs molality and calculate the x axis intercept. This is the solution concentration in the potato.

Questions:

a. What is the difference between molality (m) and molarity (M)?

b. Based on your data, at what molality of mannitol is the water potential inside your potato equal to? Using these data and the following formula, calculate the osmotic potential of the potato.

$$\Psi_S = - CRT$$

where

C = concentration of solution in molality (the x-intercept from the regression)
R = gas constant = 0.00831 (kg * Mpa)/mol K
T = absolute temperature (K) = degrees C + 273

c. What kinds of factors might influence the results of your experiment if you were to repeat it with a different batch of potatoes? Why?

WATER POTENTIAL OF POTATO												
Group #	0.0 m		0.1 m		0.2 m		0.3 m		0.4 m		0.5 m	
	45m	1.5h	45m	1.5h	45m	1.5h	45m	1.5h	45m	1.5h	45m	1.5h
Initial wt.												
1 Wt.												
Δwt.												
Δ % wt.												
2 wt.												
Δwt.												
Δ % wt.												
3 wt.												
Δwt.												
Δ % wt.												
4 wt.												
Δwt.												
Δ % wt.												
5 wt.												
Δwt.												
Δ % wt.												
Mn Δ %												

How to calculate a Least Squares Linear Regression line.

$y = bx + a$
where b = the slope; a = the y intercept

1. Make up a table with columns for x (data), x^2, y (data), and xy

2. Sum up each column and list the summations (Σ) at the bottom of the table (Σx, Σx^2, Σy, Σxy)

3. mean $x = \Sigma x/n$; mean $y = \Sigma y/n$, n = the number of observations

4. Calculate b and a using the following equations:

$$b = \frac{\Sigma xy - \frac{(\Sigma x)(\Sigma y)}{n}}{\Sigma x^2 - \frac{(\Sigma x)^2}{n}}$$

a = mean y - b(mean x)

$$r = \frac{\Sigma xy - \frac{(\Sigma x)(\Sigma y)}{n}}{\sqrt{\left\{\Sigma x^2 - \frac{(\Sigma x)^2}{n}\right\}\left\{\Sigma y^2 - \frac{(\Sigma y)^2}{n}\right\}}}$$

r = Correlation Coefficient
This is the precision by which the dependent variable can be predicted by the independent variable.

r^2 = Coefficient of Determination
This is the fraction of the total variance in the dependent variable "explained" by the regression equation. This is a good descriptor of how clearly a straight line descirbes the relationship between the two variables.

HOW TO ANALYZE AND GRAPH YOUR MOLALITY DATA IN EXCEL

Raw Data:
Enter the molality values in the first column
Enter the % weight change date in the next columns

Means and standard deviation:
In the first row of a column after the raw data enter the following formula
=average(B5:F5) > copy and paste this formula for all rows

In the first row of the next column enter the following formula
= stdev(B5:F5) > copy and paste this formula for all rows

Calculating the slope and y-intercept of the least-squares regression line:
TOOLS > DATA ANALYSIS > REGRESSION > OK

Input Y Range:
Click on spreadsheet icon > click and drag down the Mn % Wt change column > Close
Input X Range:
Click on spreadsheet icon > click and drag down the Molality column > Close

Click on Output Range > type in cell location where you want the regression output
(ex: A12)

y = bx + a
b = slope (X variable in output) = -43.8 a = y-intercept = 12.8

Creating and inserting x-y line graph
INSERT > CHART > LINE > NEXT

Data Range > click on icon > click and drag across y data
Category (X) axis labels > click on icon > click and drag down x data column
NEXT > On this dialog you can add Lables, Titles, etc.
Category (X) axis: Molality
Category (Y) axis: % Wt. Change
FINISH

Click on Graph and drag and drop where you want it on your spreadsheet.

Potato Water Potential Fall 2005

Raw Data

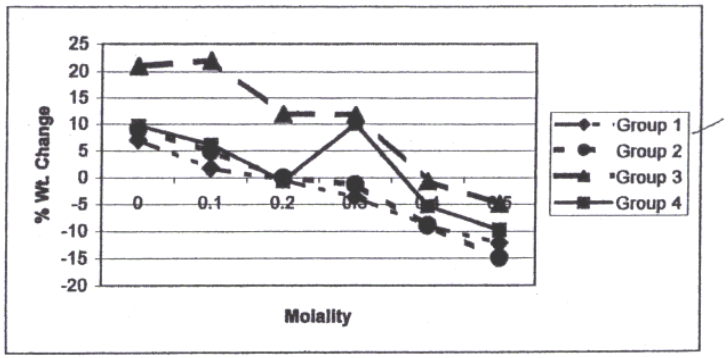
Molality	%wt.Change Grp 1	Grp 2	Grp 3	Grp 4
0	7	9	21	9.7
0.1	1.7	4.88	22	6.2
0.2	-0.42	0	11.9	-0.7
0.3	-3.7	-1.3	11.8	10
0.4	-9	-8.97	-0.71	-5.4
0.5	-12.2	-14.94	-4.95	-9.8

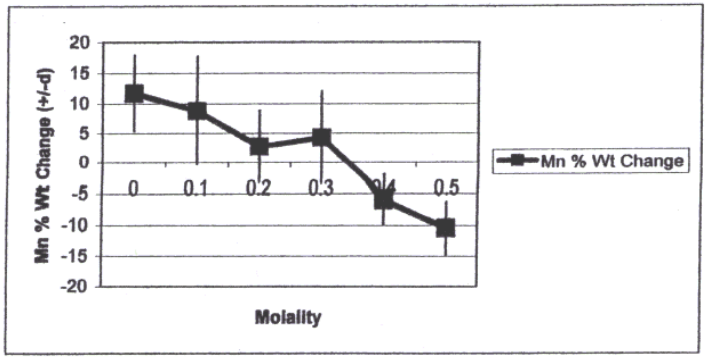
=average(B5:F5)

Mn % wt Change	StdDev
11.675	6.3211
8.695	9.0689
2.695	6.1434
4.2	7.8328
-6.02	3.9227
-10.473	4.2384

=STDEV(B5:F5)

INSERT > CHART > LINE





INSERT > CHART > LINE

To get error bars:

Right Click on Line
↓
Format Data Series
↓
Y error bars
↓
Custom
↓
Enter cell block where Standard Deviation values are listed

SUMMARY OUTPUT

Regression Output

Regression Statistics	
Multiple R	0.961678
R Square	0.924824
Adjusted R	0.90603
Standard E	2.613315
Observatio	6

ANOVA

	df	SS	MS	F	ignificance F
Regressior	1	336.0665	336.0665	49.20867	0.002175
Residual	4	27.31767	6.829417		
Total	5	363.3842			

	Coefficients	tandard Err	t Stat	P-value	Lower 95%	Upper 95%	ower 95.0%	Upper 95.0%
Intercept	12.75095	1.891379	6.741617	0.002523	7.499643	18.00226	7.499643	18.00226
X Variable	-43.8221	6.247019	-7.01489	0.002175	-61.1666	-26.4776	-61.1666	-26.4776

y=bx+a
b= -43.8
a = 12.8

if y=0
x=-a/b=0.29

solute potential = -CRT
C= **0.29** molal
R= 0.00831 (kg*(MPa)/mol K
K = 20 + 273 = 293

solute potential = **-0.71 Mpa**

You type this

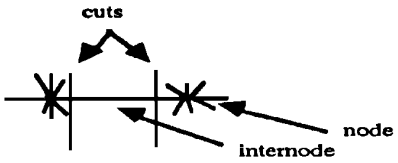
PLANT MICROPROPAGATION: ADVENTITIOUS GROWTH, NODAL PROPAGATION AND CYTOKININ EFFECTS IN PARROT FEATHER, *Myriophyllum spicatum*

Myriophyllum spicatum is an aquatic plant that grows profusely in lakes. It is actually considered a nuisance plant, rivaling *Hydrilla* spp. in the problems it causes. ***M. spicatum*** is a problem all over North America. It is an introduced plant, probably from Europe (its other name is Eurasian milfoil). ***M. spicatum*** grows quickly, and competes with native aquatic plants for light and nutrients. Generally, ***M. spicatum*** wins these competitions. It is not a good replacement for native plants, as it does not offer the same habitat quality as that of the native aquatic plants. ***M. spicatum*** has tremendous regenerative potential. Small fragments carried from one lake to the next can result in new infestations. As part of this lab, consider the use of plant micropropagation as a research tool to demonstrate the regenerative potential of this species.

Objective: The objective of this lab is to demonstrate the effects of explant type and cytokinins on micropropagation of a plant via adventitious growth. Students will compare plant production from this kind of growth to that of nodal growth for the same species.

Stage II Cultures, Adventitious Growth. Before coming to lab, students should find out what adventitious growth is. Students should consult a basic botany text to gain a clear idea as to the definition of adventitious growth.

For this experiment, we will use the aquatic plant, ***Myriophyllum spicatum.*** Sterile plants should be removed from the culture tubes and placed on a sterile surface (petri plate). Internode segments (the tissue between the nodes) are excised and placed horizontally in an experimental medium and control medium.



The experimental medium is composed of MS salts and vitamins, 2 mg/l 2iP, and 3% sucrose, pH 5.7 with 0.8% agar. The cytokinin is eliminated from the control medium. Both media are in scintillation vials.

Each group should prepare 3 replicates using each type of medium. After one week, the numbers of new shoots on the segments in the 2 media should be compared. Students can count these new growths under dissecting scopes. The class will combine data and compare the effects of the cytokinin on adventitious shoot production.

Stage II Cultures, Nodal growth. Nodal segments should be removed from the same sterile cultures used for the adventitious growth experiment. Shoot material should be cut to within 0.5-1 cm below the node, and 0.5 cm or less above the node. The basal end of each node should be inserted into the agar to prevent dessication. The medium is the same experimental medium used in Stage II Cultures, Adventitious Growth.

After one week, the numbers of new shoots and nodes on the segments should be compared. Students can count these new growths under dissecting scopes. The class will combine data and compare the effects of the cytokinin on shoot and node production.

Myriophyllum Observations		Week #:				Date:			
		Nodes-Control		Nodes + 2iP		Internodes - Control		Internodes + 2iP	
Group	Rep	Shoots	Nodes	Shoots	Nodes	Shoots	Nodes	Shoots	Nodes
1	1								
	2								
	3								
2	1								
	2								
	3								
3	1								
	2								
	3								
4	1								
	2								
	3								
5	1								
	2								
	3								

MICROPROPAGATION FROM PETIOLES WITH VENUS FLYTRAPS

Venus fly traps *(Dionaea muscipula)* are among North Carolina's most famous plants. They grow in acid, nitrogen deficient soils. The trap is a modified leaf that is supported by a petiole attached to the rhizome. Poachers threaten wild populations of Venus fly traps. A number of North Carolina horticulture businesses are propagating Venus fly traps by simple division or from seed. Micropropagation also represents a way to culture large numbers of a plant species that may be endangered.

Objective: Venus fly traps can regrow from "bulbs" which form on the base of the petioles. These are not botanically true bulbs, rather are a meristematic growth region. Students will observe production of new petioles and traps emerging from these bulbs. This will demonstrate micropropagation occurring from meristematically active regions in the petioles.

Stage II. Multiplication: The instructor will provide students with Stage II cultures of *Dionaea muscipula.* The students should remove the plants from the test tubes and lay them on a sterile surface. Using a set of sterile probes, the students should carefully dissect the different plantlets apart. Small clusters of two or three plants should be transferred into fresh Stage II Venus fly trap medium. This is a special medium, and contains the following ingredients:

Component	mg/liter
NH4NO3	400
KNO3	480
MgSO4 7 H20	370
CaCl2 2 H20	440
NaH2PO4 H20	380
FeSO4 7 H20	55.7
Na2 EDTA	74.5
MnSO4 H20	16.9
ZnSO4	8.6
H3BO3	6.2
KI	0.3
CoCl2 6 H20	0.025
CuS04 5 H20	0.025
Na2MoO4 2 H20	0.250
I-Inositol	100
Adenine sulfate	80
2iP	2
Thiamine Hcl	0.4

3% sucrose, 0.8% agar, pH of medium is 4.5

Students should compare this medium with that of MS medium of Table 1. How is the Venus fly trap medium different and why (a hint- think of the plant's habitat). Within a few weeks, students should see new plants forming from the bases of the petioles. We will record the number of shoots and roots in each culture media weekly.

Stage III. Rooting: Larger plants from Stage II cultures can be transferred to a rooting medium of half strength MS medium with 3% sucrose, 0.8% agar, pH 4.5. There should be 3-4 plants in a cluster, and one cluster per tube. After these plants root, they can be transferred to potting soil and acclimated in Stage IV greenhouse conditions.

Venus Fly Trap Observations	Week #:			Date:		
	Shoot Multiplication Media				Rooting Media	
Student Name	Shoot #	Root #			Shoot #	Root #

MICROPROPAGATION OF BASAL MERISTEMS - *HOSTA*

Hosta is an important ornamental plant used in landscaping, especially in North Carolina. There are a number of species of *Hosta,* which is also the common name of the plant. This plant can be rapidly propagated from meristematic regions in the base of the shoots.

Objective: A number of plants grow meristematic regions in the base of the shoots. For some plants, multiplication of basal shoots provides an effective method of micropropagation. Culture conditions lead to a rapid multiplication of the numbers of basal shoots, which in turn can be subcultured or rooted. Micropropagation of *Hosta* is an excellent demonstration of rapid multiplication from basal meristems.

Stage II: Multiplication.

You will be provided with vigorous *Hosta* cultures. When you remove the plant from the culture tubes, you should carefully separate out the new basal shoots located at the base. The easiest way to do this is to cut longitudinally. The cuts can produce a number of new shoot-clusters. Students should place 1 cluster in a tube of multiplication medium. This medium is based on MS salts, 0.5 mg/L IAA, 0.1 mg/L BA (5:1 auxin:cytokinin), 3% sucrose, MS vitamins, casein hydrolysate and 0.8% agar, pH 5.7. Count the number of shoots.

Each week you should look for new foliar production of shoots. You should also see the production of new basal shoots occurring along the base of the inoculated shoot.

Stage III: Rooting.

Basal shoots portions should be placed on rooting medium. This medium consists of half strength MS salts, 0.5 mA IAA (5:0 auxin:cytokinin), 3% sucrose, MS vitamins, 0.8% agar, pH 5.7. Within several weeks, the plants will generate new shoots and produce multiple roots. These will be counted each week.

Greenhouse Trials.

After roots have been produced (3-4 weeks) students should transfer newly rooted plants from test tubes into pots with potting soil. For comparisons, you will be given some Stage II cultures to also try rooting. The potted plants should be taken to the greenhouse and put under the mist system.

Hosta Observations	Week #:		Date:			
	Shoot Multiplication Media				Rooting Media	
Student Name	Shoot #	Root #			Shoot #	Root #

ORGANOGENESIS OF TOBACCO, *NICOTINA TABACUM*

Tobacco is a major model system in plant biotechnology, much like *E. coli* is for microbiology. Tobacco demonstrates organogenesis very easily. This is also a good exercise to investigate the effects of different ranges in auxin and cytokinin concentrations on plant development.

Objective: To demonstrate the process of organogensis from callus cultures and the effects of plant growth regulators on organogenesis. Organogenesis is the process where new plant organs differentiate from a seemly undifferentiated mass of tissue, the callus. In fact, a look at calli will reveal that they are themselves composed of multiple cell types.

Organogenesis 4-6 weeks after Callus induction lab

You began the process of callus induction by placing tobacco pith on a callus induction media. You will use your calli or those supplied by your instructor to examine the effects of different auxin:cytokinin ratios on shoot and root proliferation from callus.

The three media we will test are all based on the same formulation as the callus induction medium, but the ratios and concentrations of auxin (IAA) and cytokinin (kinetin) have been varied:

1.) Shoot induction media: 0.03 mg IAA/l: 1.0 mg kinetin/l (1:3 auxin:cytokinin)

2.) Root induction media: 3.0 mg IAA/l: 0.02 mg kinetin/l (150:1 auxin:cytokinin)

3.) Callus media 2.0 mg IAA/l: 0.2 mg kinetin/l (10:1 auxin:cytokinin)

Students should use good sterile technique. Remove the calli from the petri plates, and place one callus on each of the three media. Recap the tubes and seal with Parafilm.

Students should note the changes that occur in the tobacco calli over the periods of the next several weeks. In particular, note which kinds of cells seem to give rise to the new organs.

Tobacco Callus - Observations								
Group	Media	Week 0	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
1	Shoot							
	Root							
	Callus							
2	Shoot							
	Root							
	Callus							
3	Shoot							
	Root							
	Callus							
4	Shoot							
	Root							
	Callus							
5	Shoot							
	Root							
	Callus							

PHOTOSYNTHETIC RESPONSES OF MARINE ALGAE CULTURES EXPOSED TO VARYING SALINITIES

Objective:
To compare the photosynthetic rates of a local coastal algae to hypo- and hyper-salinity conditions.

Backround:
The class will be provided with thalli of a local macroalgae in GA-7 culture flasks. Today we will compare photosynthetic rates as a function of salinity in these plants. This should provide some mechanistic understanding of the effect of salinity on growth.

Experimental Procedure:
Measure the number of nodes and branches for each plant. Each group will prepare five BOD bottles, one of each of the experimental treatments. These treatments are:
1) 10 psu IO 2) 20 psu IO 3) 30 psu IO 4) 40 psu IO 5) 30 psu IO no plant (Light Blank)

Carefully open the numbered BOD bottle. Insert the oxygen electrode, turn the salinity calibration knob to your treatment salinity, start the stirring motor, and take an initial oxygen concentration reading after the meter equilibrates. Record this reading and the BOD bottle number. Transfer the plant from the GA-7 flask to a paper towel to blot off excess water, quickly place in a tared weigh pan and record the fresh weight. Then insert it into the BOD bottle. Do this over a sink. Carefully replace the tapered glass stopper, ensuring that there are no air bubbles in the bottle. Place each BOD bottle in the water-filled tray outside in the bright shade sequentially, at 2 minute intervals. Record the time.

After 1-2 hours, sequentially (2 min intervals) remove the plants from your BOD bottles. Carefully remove the algae, spilling as little medium as possible, rinse in tap water and place in a weigh pan with your group number and the treatment salinity. Then, re-stopper your bottles. When you are ready to measure the oxygen production, remove the stopper from the bottle, and insert the oxygen probe, adjust the salinity calibration knob to your salinity, and start the stirring motor on the probe. After the meter equilibrates, read the oxygen concentration. The plants will be placed in a drying oven to obtain g dry weight of material in each BOD bottle.

To determine oxygen production from photosynthesis, subtract the initial oxygen concentration from the oxygen concentration after one hour and then substract the mean del oxygen value from the Light Blank bottles (this corrects for microbial oxygen consumption/production). Photosynthetic rates will be expressed as mg oxygen per g dwt per hour by dividing the corrected oxygen production/consumption by the dry weight of the plant.

$[(\text{Final } O_2 - \text{Initial } O_2) - (\text{Blank Final } O_2 - \text{Blank Initial } O_2)] / 2 \text{ h} / \text{ g dwt} = \text{mg } O_2 \text{ g dwt}^{-1} \text{ h}^{-1}$

The class will combine data. Each student will plot mean photosynthetic rates as a function of salinity and discuss the significance of this relationship in a one-page summary.

Photosynthesis versus Salinity (O_2 mg/L)
Species: Date:

Group	10 ‰		20 ‰		30 ‰		40 ‰		Blank 30 ‰	
	t=0	t=	t=0	t=	t=0	t=	t=0	t=	t=0	t=
1										
2										
3										
4										
5										

PLANT PHYSIOLOGY DRY WEIGHT DATA						
DATE	TRTMNT	TISSU	PAN #	PAN+TIS wt	PAN wt.	TISSUE wt

BIO355 F03 Myriophyllum photosynthesis Lab

Raw Data

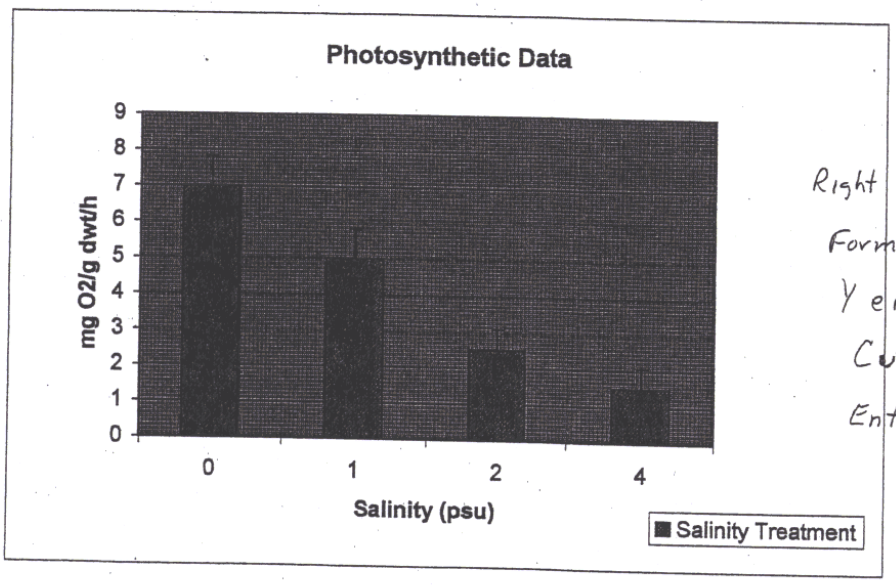
sal	O2rep0	O2rep1	O2rep2	O2rep4
0	7	5	3	1
1	8	4	3	2
2	6	5	2	1
4	7	6	2	2

Copy & Paste from Descriptive Statistics Output

Mn	SD
7	0.82
5	0.82
2.5	0.58
1.5	0.58

Tools > Data Analysis > Descriptive Statistics

*	Mean	7.00	5.00	2.50	1.50
	Standard Error	0.41	0.41	0.29	0.29
	Median	7.00	5.00	2.50	1.50
	Mode	7.00	5.00	3.00	1.00
*	Standard Deviation	0.82	0.82	0.58	0.58
	Sample Variance	0.67	0.67	0.33	0.33
	Kurtosis	1.50	1.50	-6.00	-6.00
	Skewness	0.00	0.00	0.00	0.00
	Range	2.00	2.00	1.00	1.00
	Minimum	6.00	4.00	2.00	1.00
	Maximum	8.00	6.00	3.00	2.00
	Sum	28.00	20.00	10.00	6.00
	Count	4.00	4.00	4.00	4.00



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Right Click on small square
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Enter cell block where SD values are listed

PIGMENTS

Objectives: In this exercise we will determine the chlorophyll *a* (and *b*) concentrations in the algae that we using for photosynthesis measurements. By determining the fresh wt of the tissues used for pigments and photosynthesis, we can determine the percent dry weight and use the pigment levels we determine here to express photosynthesis on a chlorophyll *a* basis, in addition to a dry wt basis.

Background: Any physiological process that is driven by light requires pigments. Plants have a variety of pigments - some photosynthetic and some non-photosynthetic (e.g., flavonoids - sunscreens). The primary photosynthetic pigment is chlorophyll *a*. Accessory pigments include other chlorophylls (*b, c,* and *d*), carotenoids (carotenes and xanthophylls) and the phycobilins. We can determine what wavelengths of light pigments absorb by examining their absorption spectra. Absorption spectra are also used to quantify the amounts of a particular pigment by using its molar extinction coefficient, which is the amount of light absorbed by one mole of the pure pigment at a particular wavelength. These coefficients vary as a function of wavelength and the solvent used to extract the pigment from the plant. Extraction involves grinding the plant tissue in a solvent in which the pigments are soluble then centrifuging the extract to clear the solvent of debris so that the spectra of the solubilized pigment can be read in a spectrophotometer.

Spectrophotometers emit light fractionated into its component wavelengths by a prism or grating. A series of slits to refine the light into monochromatic bands and a detector measures the amount of light transmitted by the sample. The mathematical relationship between the concentration of a substance and absorbance of monochromatic light is provided by the Beer-Lambert Law [$\log I_0/I = kcL$ where I_0 is the original light intensity, I is the intensity of light after passing through the sample, k is the molar extinction coefficient, c is the concentration of the sample and L is the optical thickness of the sample in cm]. $\text{Log } I_0/I$ is also known as optical density (O.D.) or absorbance (A).

Methods:

Weigh out approximately 0.2 g of blotted fresh tissue and record the fresh weight. Cut into small pieces and place the tissue in a cold mortar and pestle and add 2 ml 90% acetone. Grind the tissue until no large fragments remain. Pour this extract into a chilled 15 ml centrifuge tube. Rinse the mortar and remaining extract into the tube with the wash bottle and a minimal volume of acetone, keeping the total volume to 10-12 ml. Record the volume and keep the tubes on ice. We will centrifuge all the tubes at once (4500 rpm for 10 min). After centrifuging keep the tube on ice in the dark until we measure the supernatant on the spectrophotometer. The spec will first be blanked with a cuvette filled with 90% acetone to correct for any absorbance by the solvent. The samples will then be scanned and the absorbances at 647, 664 and 750 (scattering correction) will be recorded.

Pigment quantification:

$\mu g \text{ Chl } a/g \text{ dwt} = [11.93(A_{664}-A_{750}) - 1.93(A_{647}-A_{750})]*\text{Acetone (ml)}/[(\text{fresh wt}) *(\% \text{ dry wt})]$

$\mu g \text{ Chl } b/g \text{ dwt} = [20.36(A_{647}-A_{750}) - 5.50(A_{664}-A_{750})]*\text{Acetone (ml)}/[(\text{fresh wt}) *(\% \text{ dry wt})]$

PHOTOSYNTHESIS VERSUS IRRADIANCE - A FLUOROMETRIC APPROACH

Objective:

To determine the light intensity dependence of PSII quantum yield and light saturation of electron transport rate of sun-adapted and shade-adapted plants using a pulse-amplitude modulated fluorometer.

Backround:

During photosynthesis CO_2 is reduced to carbohydrate with the aid of two photosystems operating in series, the terminal electron donor being H_2O which is oxidized by PSII. Thus, electron flow in PSII corresponds to the overall photosynthetic electron flow. There is a quantitative relationship between chlorophyll fluorescence and the efficiency of photosynthetic energy conversion. With increasing photosynthetically active radiation (PAR) PSII can potentially pump more electrons in the electron transport chain provided all the PSII centrers are open. When the rate of quanta absorption exceeds the capacity of the dark reactions, electrons accumulate at the acceptor side of PSII and Q_A becomes partially reduced. This results in a decrease of PSII quantum yield, and eventually electron transport rate (ETR) will reach saturation. With the help of fluorescence, the relative quantum yield of PSII and the effect of irradiance on electron transport can be determined in a nondestructive manner.

Experimental Procedure:

We will determine quantum yield vs PAR and ETR vs PAR using a Waltz Mini-PAM fluorometer for greenhouse-grown plants that have been sun-adapted or shade-adapted and that have been placed in high light and darkness for at least a 30min acclimation period. Since we have only one PAM fluorometer we will place the plants in the appropriate light treatment at 15 minute intervals. Three groups will measure sun-adapted plants (3 replicate leaves/plant) and the other two groups will measure shade-adapted plants (3 replicate leaves/plant).

Make sure the Mini-PAM is turned on and with nothing in the Leaf-Clip Holder that the F value is 0-3. If not then auto-zero. For each plant, the Leaf-Clip Holder will be attached to a mature leaf mid-way along the leaf between the mid-rib and leaf margin. Adjust the tripod so that the leaf is held in the Leaf-Clip holder in nearly its natural position (minimize bending). When the Leaf-Clip holder is secure, press + and ^ simultaneously to begin the rapid light curve program. Note the time when you started your curve. You will see 9 flashes of light and the entire photosynthesis versus irradiance curve measurement should take less than 1.5 minutes. When the last light level measurement is complete, press MEM on the Mini-Pam and it will show the last Yield (Y) ETR (E) and Light level (L) record these values in a table (see below) recording on the last line first and use the ^ key on the Mini-PAM to read the next 8 values.

The class will combine data to obtain mean Yield, ETR and Light values for the replicate plants of each treatment (2 treatments total). Each student will plot the mean Yield and ETR versus Light values for the two experimental treatments (sun versus shade-adapted) and discuss the significance of the resulting curves with a one page special report due next week.

$Y = (Fm-Fo)/Fm$ ETR = Yield x PAR x 0.5 x 0.84 where 0.5 assumes 1:1 PSII:PSI and 0.84 = Leaf Absorptance Factor

PAM FLUOROMETER DATA SHEET											
Species:			Treatment:			Date:			Group:		
Leaf 1			Leaf 2			Leaf 3			Mean		
Y	ETR	L	Y	ETR	L	Y	ETR	L	Y	ETR	L