Going Green "Under the Hood" - the Sterile Hood

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Abstract

There is a diverse plethora of plants in our world. Human destruction, however, is causing rapid extinction to many. Plant tissue culture (PTC) is a technique that allows clones of plants to be reproduced quickly and at an exponential rate. The objective of this project is to exemplify the ability to produce thousands of plants from one original plant using micropropagation, or PTC. The procedures can be performed anywhere from a kitchen-based lab to a multi-million dollar one, as long as the proper equipment is provided. Tissue culture ensures rapid growth of healthy and sterile plants. Plants require 17 essential elements to maintain proper growth and development and are provided as Murashige and Skoög salts (MS salts), in the amounts required for an "average" plant growth. All elements, supplements (except sucrose), and hormones are provided as a pre-mixed powder to constitute 1000 milliliters of either MSO (no hormones), MSS (shoot media), or MSR (rooting media). In this culture, I will be growing cauliflower on an MSS (Stage 2) media, demonstrating how inexpensive and fast micropropagation is.

Introduction

Tissue culture, or micropropagation is an area of many facets that anyone from the curious gardener in their home kitchen to a prominent scientist in a multi-million dollar lab can perform. It is involved with botany, chemistry, genetic engineering, microbiology, molecular biology, and even food science, just to name a few. Utilizing tissue culture is a way to grow healthy and clean plants by cloning a particular plant, or even by manipulating genes and transferring desirable characteristics.

Even in the booming complexity of the botany field, tissue culture, which is not known by many people, can actually be a simple process. The first step is to obtain a part of the "mother plant," which will be sterilized and grown *in vitro* to provide thousands of identical copies of the original mother plant. It can be anything from a leaf, a stem, a root, or an apical meristem. That piece of plant is called an *explant*, is sterilized with bleach, and then placed in a test tube or Petri dish, an environment free of any microorganisms, and fed a medium that contains the proper nutrients for it to grow. Overall, tissue culture is comprised of 4 main stages:

Stage 0: The Mother Plant
The Mother Plant is any plant found in nature. This stage involves sterilizing the *explant* in bleach for 15 minutes and rinsing with sterile water.

Stage I: Initiation
The next step is to take the *explant* and insert it into a media called MSO, which contains no hormones. This will produce "callus," or undifferentiated parenchyma cells. The MSO media causes the callus to increase in size and can then be divided into at least **10** separate petri dishes to receive Stage 2 treatment.

Stage 2: Multiplication
The 10 sections of "callus" will then be inserted into a media called MSS. It contains hormones called cytokinins which cause shoot growth. The 10 MSS cultures can now each be divided to 10 separate petri dishes to receive Stage 3 treatment. That is **100** petri dishes!

Stage 3: Rooting
After shoots have grown, the plant will be transferred to a media called MSR to promote root growth. When the root growth is complete, small mini plants have developed. Each of the 100 Stage 3 cultures can again each be divided into 10 separate cultures. This will result in **1,000** final plants that are exact copies of the original "mother plant," only within about 8 short weeks, depending on the species of plant.

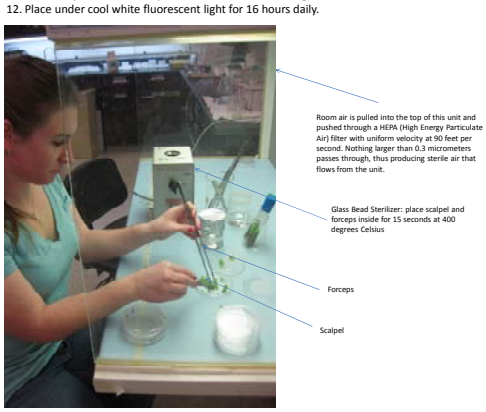
Stage 4: Hardening Off
The last stage is returning the plant to where it came from: the ground. The plant can now be transplanted into a greenhouse.

Materials

- Mother Plant
- Scalpel
- 70% Ethanol
- Glass Bead Sterilizer
- 1-liter Erlenmeyer flask
- Sink
- 30 g/L sucrose
- Petri Dishes
- 2.5 mg/L "kinetin" Cytokinin
- Hot plate/ stirrer and magnetic stir bar
- Murashige & Skoög Salt Base Components
  - NH_4NO_3
  - $CaCl_2$
  - KH_2PO_4
  - H_3BO_3
  - $ZnSO_4 \cdot 7H_2O$
  - $Na_2MoO_4 \cdot 2H_2O$
  - $CoCl_2 \cdot 6H_2O$
  - KNO_3
  - $MgSO_4$
  - FeNaEDTA
  - $MnSO_4 \cdot H_2O$
  - KI
  - $CuSO_4 \cdot 5H_2O$
- Autoclave
- Forceps
- Bleach
- Light Bank
- Distilled water
- pH meter
- 8g/L agar
- Scale
- Sterile hood - model "Laminar Flow" with HEPA filter

Methods

1. Dissolve one packet of MS Salt into 800 mL of deionized water. Mix well. Add 30 grams of sucrose. When completely dissolved use 1 N Sodium Hydroxide or 1 N Hydrochloric Acid to adjust the pH. A pH of 5.7 is recommended for agar cultures. Add additional water to bring the volume to 1000mL. Add 8 grams of agar.
2. Insert a stirring bar and place the flask on a hot plate and let the mixture boil until the agar has melted.
3. Dispense into petri dishes or test tubes.
4. To sterilize the media, cover and process in an autoclave for 15 minutes at 18 pounds of pressure at 250 degrees Celsius.
5. At the same time, sterilize dI water to be used for explant cleaning.
6. Once media has cooled, break a cauliflower (curd) into pieces. These will serve as the explants.
7. Spray them with 70% ethanol.
8. Let them soak for 10 minutes in 20% bleach.
9. To rinse, let the pieces soak in sterile deionized (dI) water for 5 minutes.
10. Cut 1/8 of an inch off of the base to remove the bleach damaged area.
11. Stand it up like a tree and push it into the MSS Shooting Media.
12. Place under cool white fluorescent light for 16 hours daily.



Results

Table 1 shows that after 5 weeks of growing in MSS media, each of the cauliflower explants approximately doubled in both length and width.

Table 1. Measurements of Width and Length for Explants A, B, and C

Time	Width (mm): Explant A, (B), (C)	Length (mm): Explant A, (B), (C)
Week 0: 2/1/2012	7, (11), (17)	10, (11), (17)
Week 1: 2/8/2012	11, (13), (21)	11, (12), (24)
Week 2: 2/13/2012	16, (14), (24)	16, (13), (31)
Week 3: 2/22/2012	17, (15), (26)	17, (17), (32)
Week 4: 2/29/2012	21, (17), (28)	19, (17), (33)
Week 5: 3/7/2012	21, (21), (30)	22, (19), (42)

Figure 1. Correlation of plant growth in width in relation to time spent in MSS media

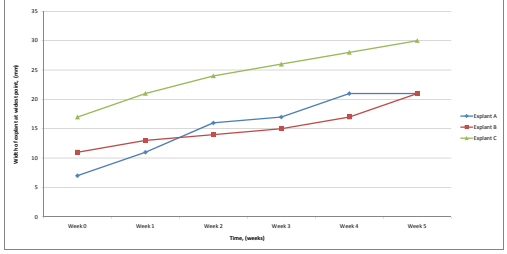
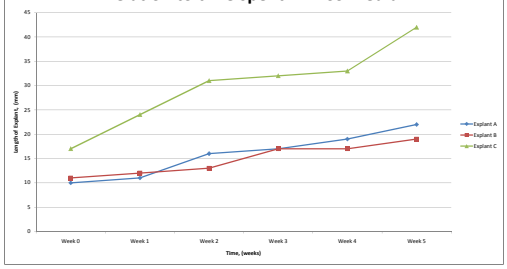
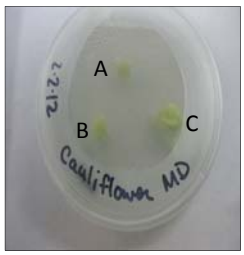
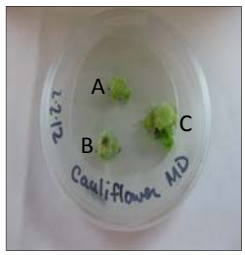


Figure 2. Correlation of plant growth in length in relation to time spent in MSS media

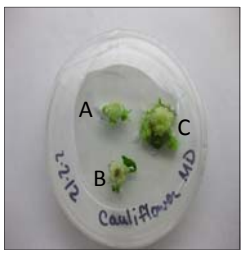




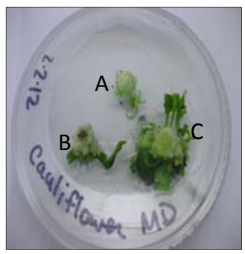
Week 0



Week 1



Week 2



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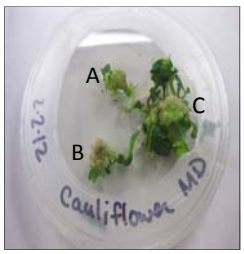
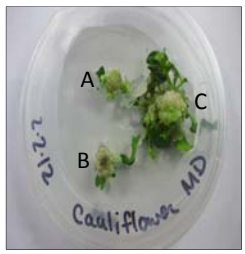


Week 4



Week 5

Conclusion

Since we established that micropropagation indeed works to double cauliflower within five weeks, we have verified that it is a cheap and fast way to multiple the number of plants. The nutrients present in the media are suited to an individual plant's needs so that the plant growth increases rapidly. During my tissue culture of cauliflower, anthocyanins, the purple color seen on the cauliflower, developed within one week. My plants grew successfully each week, both in width and height resulting in growth doubling overall. If I had continued my project, it would have been time to switch the cauliflower to MSR (Stage 3) media, as the plants had used the agar media in the MSS plates. However, the parafilm covering the sides of the Petri dishes became loose and did not cover the gap in between the Petri plate and lid. Therefore, this created an opening in which microorganisms were allowed to enter inside the Petri dishes. The last week that I took measurements, I found contamination because of this reason.

Discussion

Tissue Culture has a plethora of advantages, including:

- The ability to make a copy of a plant that has a favorable trait such as good fruit or pretty flowers. This is different than using seeds, because seeds have genetic variation. Tissue culture involves making an exact copy of the same plant. This is beneficial especially in cases involving rare plants. Tissue culture makes it easy to quickly reproduce healthy plants that are endangered.

- Producing mature plants fast: Plants can grow at an exponential rate and generally plants *in vitro* grow faster than a plant sitting in nature. One explant can be divided into 10 for Stage 2, 100 for Stage 3, and produce 1000 plants to be planted! An example of this can be seen in Figure 3.

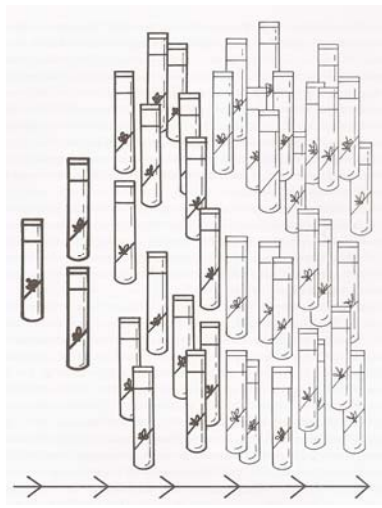


Figure 3. The exponential growth of clones through tissue culture

- Tissue culture produces many plants quickly without natural pollinators or seeds: Some seeds take a long time to germinate or some are inviable. Also, some plants, such as seedless fruits do not produce seeds.

- The ability to grow a whole plant from a small piece of the original: This is called totipotency – the ability of one plant cell to grow into an entire plant.

- Growing plants in a sterile environment protects them from diseases and pests.

- The environment can be manipulated to suit the needs of each individual plant. Light and temperature can be changed accordingly and adding sugar or hormones to media can help to mimic the environment the plant came from.

- Little care is required in tissue culture other than subculture. This saves space, time, and cost. Subculturing is dividing the plant and putting the pieces in fresh media when all of the media has been used.

- Tissue culture makes it possible to clone hard-to-grow-plants, including orchids, which are the plants that made tissue culture popular.

Acknowledgements

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