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STUDTY OF PLANT TISSUE CULTURE METHOD

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Abstract : Plant research often involves growing new plants in a controlled environment. These may be plants that we have genetically altered in some way or may be plants of which we need many copies all exactly alike. These things can be accomplished through tissue culture of small tissue pieces from the plant of interest. These small pieces may come from a single mother plant or they may be the result of genetic transformation of single plant cells which are then encouraged to grow and to ultimately develop into a whole plant. Tissue culture techniques are often used for commercial production of plants as well as for plant research.

Introduction : Tissue culture involves the use of small pieces of plant tissue (**explants**) which are cultured in a nutrient medium under sterile conditions. Using the appropriate growing conditions for each explant type, plants can be induced to rapidly produce new shoots, and, with the addition of suitable hormones new roots. These plantlets can also be divided, usually at the shoot stage, to produce large numbers of new plantlets. The new plants can then be placed in soil and grown in the normal manner.

Many types of plants are suitable for use in the classroom. Cauliflower, rose cuttings, African violet leaves and carnation stems will all easily produce clones (exact genetic copies) through tissue culture. Cauliflower florets in particular give excellent results since they can be grown into a complete plant in the basic tissue culture media, without the need for additional growth or root hormones. Green shoots are generally observable within three weeks, and roots develop within six weeks.

The most important part of this activity, however, is to maintain as sterile an environment as possible. Even one fungal spore or bacterial cell that comes into contact with the growth media

will rapidly reproduce and soon completely overwhelm the small plant piece that you are trying to clone.

Plant tissue culture was first proposed by the German Botanist Golliob Haberlandt in 1902. He is regarded as the father of plant tissues culture. He mainly worked on palisade tissue and grew them on knob's salt solution with sucrose and observed the growth of cells. Hanning (1904) excised matured embryos of crthe ucifers and successfully grew them on the mineral salt and sugar solution. The embryo culture was further developed by over back (1941). This proved to be a turning point in plant tissue culture. In 1972, Carlson and other produced the first somatic hybrid between Nicotiana gluca and N.langschorffii by fusing their protoplast. Tissue culture of first used on large scale by the orchid industry in 1950s.

Objectives

- To understand a procedure that is often used to propagate many plants of the same genetic background.
- To understand the importance of sterile techniques.
- To multiple single explant in to several thousand plants.
- To produce virus free explant.

Materials &Methods : Plant tissue culture method required below material.

1 Vial of Murashige Skoog (MS) media , 1 L sterile distilled water , 10 g of agar/L ,30 g sucrose/L , 1.5 L or 2 L container in which to prepare the growth medium , Your chosen plant (cauliflower, rose, African violet or carnation) ,Cutting equipment such as a scalpel blade or razor blade , small amounts of 1M NaOH and 1M HCl to adjust the pH of the media , paper towel for cutting on or sterile petri dishes if available , 60 flat bottom culture tubes with closures., 2 bottles of sterile distilled water *(purchase at the grocery store)* , Beaker or jar in which to wash the plant material , Hormones such as BAP, Pressure cooker ,Glass aquarium or box lined with plastic , Plastic sheet to cover the top of the aquarium , 2 or 3 beakers or jars of sterile water , Bleach sterilizing solution - dilute commercial bleach (5-6% sodium hypochlorite) to a final concentration of 1-2% sodium hypochlorite in distilled water in a large beaker or jar, A well-lit area away from direct sunlight or use full-spectrum gro-lights, to stimulate growth and root development, respectively, Detergent-water

mixture - 1ml detergent per liter of water ,Adhesive tape ,10% Bleach in a spray bottle , 70% alcohol in a spray bottle , Forceps or tweezers ,Gloves.

- **Method Preparation and sterilization of growing medium** : These steps will make 1 L of growth medium which is enough to prepare about 65 growing tubes.
- Dissolve the MS mixture in about 800 ml of distilled water. Stir the water continuously while adding the salt mixture. Add 30 g sugar and stir to dissolve. Adjust pH to 5.8 using 1M NaOH or 1M HCl as necessary while gently stirring. Add distilled water to make the total volume up to 1 L.
- Weigh out 10 grams of agar and add it to the MS solution. Heat the solution gently while stirring until all the agar has dissolved.
- Pour the still warm medium into the polycarbonate tubes to a depth of about 4 cm which will use about 15ml of media per tube.
- Place the tubes (with lids sitting on the tubes but not tightened) in a pressure cooker and sterilize for 20 minutes. Cool the pressure cooker, then remove the tubes and tighten the lids. Alternatively the tubes can be placed in boiling water for 30 minutes, but make sure that none of the water is able to enter the tubes.
- **Method of Preparation of a sterile transfer chamber and equipment**

A classroom transfer chamber can be made from a clean glass aquarium turned on its side. Scrub the aquarium thoroughly with a 30% bleach solution, making sure that you wear gloves and do not inhale the fumes. Rinse with sterile distilled water, turn upside down on a clean counter or paper towels and allow to dry. Cut holes in a clean plastic sheet to allow arms to reach into the chamber and reinforce the cut edges with tape if necessary. Tape the clean plastic sheet over the open side of the aquarium making sure that the arm holes are located at a convenient height. Plastic sleeves could also be fitted to these holes if you wish to make it easier to prevent the entry of airborne spores into the chamber. The finished aquarium chamber can be sterilized by spraying with 10% chlorox bleach just prior to each use and drying with sterile paper towel. Wrap the forceps, scalpels, razor blades, paper towel and gloves (rubber or surgical) in aluminum foil, seal with tape and sterilize by processing

them in a pressure cooker for twenty minutes. These items can also be sterilized by placing in an oven at 350oF for 15 minutes. You can wrap each item separately or put together a "kit" so that each student will have their own sterile equipment to use.

Alternatively the forceps and blades can be sterilized by dipping in 10% bleach and then rinsing in sterile water, or dipping in alcohol and then placing in a flame, although this is not recommended for use in crowded classrooms. If you choose to dip in bleach and rinse in sterile water, it is best if fresh solutions are available for each 3-4 students since the water can easily be contaminated if care is not used. These liquid containers should only be opened once they are inside of the sterile chamber.

- **Method Plant preparation :**Your plant material must first be surface sterilized to remove any bacteria or fungal spores that are present. We aim to kill all microorganisms, but at the same time not cause any adverse damage to the plant material.
- Cauliflower should be cut into small sections of florets about 1 cm across. If using a rose or other cuttings, cut the shoots into about 5 to 7 cm lengths. Whole African violet leaves can also be used.
- Wash the prepared plant material in a detergent-water mixture for about 20 minutes. If trying hairy plant material scrub with a soft brush (toothbrush). This will help remove fungi etc., and the detergent will help wet the material and remove air bubbles that may be trapped between tiny hairs on a plant.
- Transfer the washed plant material to the sterilizing chlorox solution. Shake the mixture for 1 minute and then leave to soak for 10-20 minutes. Carefully pour off the bleach solution using the lid to keep the plant tissue from coming out and then carefully cap the container.

4. Method Transfer of plant material to tissue culture medium : Use the sterile gloves and equipment for all of these steps :

- Place the plant material still in the chlorox bleach sterilizing container, the containers of sterile water, the sterilized forceps and blades, some sterile paper towel to use as a cutting surface and enough tubes containing sterile medium into the sterile aquarium. The

outside surfaces of the containers, the capped tubes and the aluminum wrapped supplies should be briefly sprayed with 70% alcohol before moving them into the chamber.

- The gloves can be sprayed with a 70% alcohol solution and hands rubbed together to spread the alcohol just prior to placing hands into the chamber. Once students have gloves on and sprayed they must not touch anything that is outside of the sterile chamber.
- Carefully open the container with the plant material and pour in enough sterile water to half fill the container. Replace the lid and gently shake the container to wash tissue pieces (explants) thoroughly for 2-3 minutes to remove the bleach. Pour off the water and repeat the washing process 3 more times.
- Remove the sterilized plant material from the sterile water, place on the paper towel or sterile petri dish. Cut the cauliflower into smaller pieces about 2 to 3 mm across. If using rose cut a piece of stem about 10 mm in length with an attached bud. The African violet leaf can be cut into small squares about 1-1.5 cm across. Be sure to avoid any tissue that has been damaged by the bleach, which is apparent by its' pale color.
- Take a prepared section of plant material in sterile forceps and place into the medium in the polycarbonate tube. Cauliflower pieces should be partly submerged in the medium, flower bud facing up. Rose or other cuttings should be placed so that the shoots are level with the medium surface. The African violet leaf pieces should be laid directly onto the medium surface.
- Replace the cap tightly on the tube.

The small explant develops callus which then produces shoots a few weeks after being placed into tissue culture media seen in Figure 1.

- **Method of Growing the plants**
- The tubes containing plant sections may be placed in a well-lit area of the classroom although not in direct sunlight. The shoots will probably grow more quickly if the explants are placed under fluorescent or grow-lights to provide at least 12 hours of light per day. The aquarium can be used as a growth chamber with the lighting about 8-10" overhead. This will also help maintain a more regular and warm temperature. Ensure that the temperature does not go over 28oC. New shoots should develop within 2 weeks, and should be well advanced in 3 to 4 weeks. Check the tubes daily and discard any that show signs of infection (before discarding first sterilize in the pressure cooker or add bleach into the tube).
- Roots can appear within 6 weeks on cauliflowers. The roses, African violet and other cuttings will need to be moved into rooting media for roots to properly develop. This transfer to the second, rooting media must be conducted under the same sterile conditions as at the initiation of the culture. All necessary equipment and the aquarium should be set up as before and properly sterilized.
- Working inside the sterile aquarium chamber, remove the cap from the culture tube. There will usually be several shoots that have arisen from each explant. These shoots should be carefully separated by gently removing the whole explant from the media with sterile forceps and then separating the shoots by gently pulling them apart using two pairs of forceps. Each shoot should then be placed into a tube of rooting media and the bottom

of the shoot pushed into the media so that good contact is made. The cap is replaced and the shoots are then allowed to grow as in step 1 until roots are formed, usually within 2-3 weeks.

- **Method of Potting the clones :**Once roots are well formed the plants are ready to be transferred into soil as per below procedure :

Moving plants to pots of soil

- Each plant should be carefully removed from its tube of media and planted into a small pot containing a clean light potting mix. Gently wash off all the agar medium prior to planting. The plants will still need to be protected at this stage since they are not accustomed to the drier air of the classroom when compared to the moist environment of the tube of media.
- 2. Place all of the pots onto a tray and cover lightly with a plastic dome or tent. Place the plants in an area with 12-16 hours of light (either natural or artificial) but not direct sunlight.
- 3. After a week the cover can be gradually removed and the plants acclimated to stronger light and drier atmospheric conditions.

- 4. You now have a collection of plants in your classroom that are genetically exactly the same. You could use these plants to carry out other experimental tests knowing that one of the main variables in the experiment has been eliminated. Some of these tests could include looking at plant responses to low light levels, to drought or to saline soil conditions.

Conclusion:

Tissue culture is one of the most important part of applied biotechnology. In the coming decades the world's population will increases more and accommodation space, agricultural lands will decrease significantly global climate change is also another consideration .keeping these in mind we have to ensure a peaceful, healthy and hunger free greener world for our next generation. For doing this there is no alternate of plant tissue culture.

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