



## Horticultural Science Group

### Department of Agronomy and Soil Science

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## Plant Tissue Culture for Home Gardeners

Micropropagation is an important alternative to more conventional methods of plant propagation. It involves production of plants from very small plant parts (e.g. buds, nodes, leaf segments, root segments etc.), grown aseptically (free from any microorganism) in a container where the environment and nutrition can be controlled. The resultant plants are genetically identical to parent plants.

Whilst in a research laboratory such as that in the Department of Agronomy and Soil Science at UNE we use many high tech equipment to achieve plant production through tissue culture, it is important to note that many home gardeners and hobbyists could substitute the high tech equipment and instruments with ordinary household items.

### Items Needed for Home Tissue Culture:

- 1 - A sterile still air cabinet used to transfer plants. A fish tank on its side makes an ideal transfer cabinet. Any perspex or glass chamber with dimensions of 50 cm (length), 40 cm (height) and 40 cm (depth) could easily be made into a transfer cabinet.
- 2 - A pressure cooker for sterilisation of media, instruments, water, paper towelling etc.
- 3 - Glass jars (baby food jars are excellent) and take away food containers with lids which can withstand the heat inside a pressure cooker are ideal vessels to use.
- 4 - Scalpel and forceps
- 5 - Paper towelling or even A4 white copy paper, cut to size, can be sterilised and used for a sterile cutting surface.
- 6 - A spirit lamp containing ethanol for flaming the instruments (avoid using Methanol as it is toxic!).
- 7 - Hand held spray bottle containing 70% alcohol solution to spray the transfer chamber and other surfaces.
- 8 - Dilute chlorine solution e.g. 1/4 dilution of the household bleach (e.g. White King) for use in surface sterilisation of plant material.
- 9 - Any skin disinfectant e.g. Hibitane (obtainable from any chemist shop).
- 10 - Media (see below)

### Media Preparation

All the ingredients indicated below can be purchased using the super market, chemist and a health food shop.

- 1- Two cups of rain water
- 2- A quarter cup of sugar
- 3- Fertiliser stock: 1/2 tablespoon all purpose 10:10:10 (N.P.K.) water soluble fertiliser in 1L of water:

use one cup of stock for this recipe.

4- Inositol tablets (500 mg): 1/2 tablet

5- Vitamin tablet with thiamine: 1/2 tablet- Any multivitamin tablet may be used.

6- Agar flakes: 4 tablespoons

This is the basic media. For preparation of multiplication and rooting media add 1/2 cup of coconut milk and 1/2 teaspoon of malt. Replacing the coconut milk with 1/2 cup of green tomato puree or 1/2 cup of freshly squeezed orange juice may produce different responses. Ensure that the pH of medium is always between 5 and 6 using narrow range pH indicator tape. Adjust pH if necessary, with acid e.g. Citric acid or base e.g. bicarb soda.

Mix the ingredients in a saucepan and gently boil until the agar has dissolved, stirring continuously to stop the agar sticking and subsequently burning at the bottom of the pan. Dispense into empty glass jars, using a ladle, so that the medium is about 2 cm deep. Cover and process in a pressure cooker. Cook for 15 minutes after the pressure is reached (this will be achieved when the pressure valve starts letting steam out).

## **Sterilising Instruments and Other Items**

Forceps and scalpels can be sterilised by being wrapped in Alfoil and cooked in the pressure cooker for 15 minutes. These items can also be sterilised by being washed in chlorine solution or being dipped in alcohol and flamed.

Sterile water is needed for rinsing plant material and sterile paper towelling to be used as a clean surface to work on. The manipulation can be accomplished on the paper. When the operation is completed, the towel can be discarded and a new sterile surface selected from the sterile supply. The water can be sterilised in glass jars. Place paper inside a paper bag and cook them in the pressure cooker above the water level. The bag will be wet on completion of sterilisation. Transfer the bag to an oven set at 80 °C and allow the bag to dry inside the oven. Do not unwrap the papers until needed.

## **Sterilisation of Plant Material**

All plant material can be sterilised in diluted domestic bleach, for example White King (1/4 cup of White King + 3/4 cup of water + 1 drop of detergent- detergent acts as surfactant). Put plant pieces in a jar containing the bleach for 10-20 minutes. Agitate frequently. Discard the chlorine solution, this process will kill bacteria and fungi and sometimes some parts of the plant such as outer bud scales and softer shoots. Rinse plant pieces twice with sterile water.

## **Operations in the Sterile Cabinet**

Great care should be taken to ensure that your cultures are free from contamination. To achieve this do the followings:

- 1- Tie back your hair, roll your sleeves up and remove your watch and other jewellery. Wash your hands thoroughly with the disinfectant solution suitable for skin application. If allergic to any disinfectant wash your hands with water and wear a pair of surgical gloves.
- 2- Sterilise the inside of the cabinet by spraying with 70% alcohol and wiping dry with sterile tissue.
- 3- Collect and organise all the items you will need close to or inside the cabinet.
- 4- Working in the cabinet, take a sterilised piece of stem from the jar with a pair of forceps (do not touch the plant material with your hands). Also sterilise your instruments by dipping them in alcohol between

each manipulation and flaming them. Small pieces, 2-3 cm long with a few leaves can be cut and transferred to agar medium. If the leaves are too large either remove them or cut them to 1/3-1/2 the size. Put one piece of the shoot into each container (it is important to have only one shoot per container at this stage so that if the shoot is contaminated it cannot spread to the others). Shut the lids of the containers. Store jars at room temperature away from direct sunlight. Leave these shoots for one month.

One of these three things will happen during this time: some of the shoots will be killed by the chlorine solution and or the toxin produced by plants themselves; some will be contaminated by fungi and bacteria; or new shoots will grow very rapidly from the axils of the stems in the uncontaminated containers. Discard the dead and infested cultures.

## Shoot Multiplication

The shoots from the previous stage may have elongated during the past four weeks. They need to be transferred to the medium containing the coconut milk (coconut milk has some growth promotory properties which makes plant segments to produce more shoots) for further multiplication.

- 1- Repeat the preparation and sterilisation steps for the medium, instruments and chamber as before. Sterilise your hands as before too.
- 2- Transfer the containers of shoots to one side of the cabinet and the sterilised medium at the other side.
- 3- Use sterile paper towelling, scalpels and forceps as before.
- 4- With a pair of forceps, remove a stem from its container, and cut on the surface of the sterile paper towelling moistened with some sterile water. It is important to do all the manipulation on a damp paper towel as these plants are very soft and can desiccate readily. The separated shoots can be transferred to the new jars. At this stage, up to five pieces of plant may be put inside each container.
- 5- Store cultures as explained in the previous stage.

The multiplication stage may be repeated every four weeks until enough plants have been obtained. As a general rule shoots can multiply 3-4 folds every four weeks. The important point to note here is that high rate of contamination during this stage suggests that you are getting air borne contaminants due to poor hygiene.

## Root Formation

Once you have established enough shoots, let them grow to at least 2 cm before beginning the rooting process. Transfer shoots to the rooting medium containing coconut milk and malt. Up to five shoots may be put in each culture vessel. Store containers in their usual place as before. Roots should form within two to four weeks.

## Transfer to Potting Mix (Acclimatisation)

The operation at this stage is carried out on the open bench. The rooted cultures can be treated as follows:

- 1- Fill the pots with suitable potting mix without any fertiliser and water well. Allow to drain.
- 2- Remove the rooted plants from agar medium using a pair of forceps.
- 3- Wash off the agar thoroughly from the roots using lukewarm water.
- 4- Insert a hole in the middle of the potting mix and gently insert the roots in that hole.
- 5- Spray the foliage with a hand spray containing water. These pots can be kept inside a larger plastic containers with a glass cover, out of the direct sunlight. Gradually remove the glass cover but watch for signs of desiccation and if needed use the hand spray to spray water on the foliage.
- 6- When the roots are well established and the plants are acclimatised (this should take about 4-6 weeks), they can be given fertiliser and be treated like any other plant. It is advisable to gradually increase the light intensity for the plants too.

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