

BIO ART LAB: HARDWARE . SOFTWARE . WETWARE

RECIPE #01B: PLANT TISSUE CULTURING

MATERIALS & EQUIPMENT

- Plant matter for explant cuttings
- Micro boxes / test tubes / baby jars
- Cooker / hotplate
- Pressure cooker / autoclave - for sterilising
- Pan & spoons
- Air Purifier with hepa filters.
- Sterile transfer chamber / cabinet / fishtank
- Clean sterilise surface - tile, sterilised paper ...
- Scalpel / scissors
- Forceps / tweezers
- Burner - use ethanol for burning
- Alcohol - ethanol at 70%
- Hand / skin disinfectant
- Bleach - 1/4 dilution
- Detergent / washing up liquid
- pH indicator tape: pH balance 5.0 - 6.0

MEDIA FOR PLANT TISSUE CULTURING

Culturing media

- Sugar- 100 grams for carbon energy
- Water soluble fertiliser - 200ml: 1/2 tablespoon of all purpose 10:10:10 - N.P.K in 1L of water
- Inositol tablet (500 mg)- 1/2 tablet
- Vitamin tablet with thiamine - 1/2 tablet

or use Murashige & Skoog salts (M&S)

- Agar flakes - 100 grams
- Distilled water - 400ml
- Sterile water

Rooting and Multiplication media

Use the above media + variations of the below

- Coconut milk - 125ml
 - Malt - 5ml
 - Citric acid
 - Bicarbonate of soda
 - Orange juice
- These are growth regulators and encourage cell enlargement and division.

Adapted from a recipe from Dr. Acram Taji and the publication 'Plants from Test Tubes: Introduction to Micropropagation' by Lydiane Kyte and John Kleyn.

STAGE 1 : CULTURING

- Prep all areas to make sterile as possible.
 - Sterilise the cabinet by spraying alcohol (70 % ethanol), allow to dry.
 - Sterilise all tools needed for culturing by dipping with alcohol or spraying then flaming.
 - Sterilise water in baby jars or microbox in the pressure cooker.
 - Sterilise seeds and plant material by washing in a diluted domestic bleach solution- 1/4 bleach to 3/4 water + 1 drop detergent. Wash for 10 - 20 minutes, agitate frequently then wash in sterilised water 2 - 3 times.
- Mix the basic media recipe over heat and then add fertiliser.
- Pour about 2cm in depth into microboxes or the baby jars. Cover or close the vessels and process in a pressure cooker for 15 mins once pressure is reached.
- Carefully place one small sterile piece of plant stem or shoot with tweezers into the agar solution in the baby jars or microboxes. Work on one side of the cabinet. Make sure not to touch the top and sides. Close lids and keep in a warm and light place for about 4 weeks.

STAGE 2 : MULTIPLICATION

The explants can be multiplied once grown. This can take place every 4 weeks and the plants can be multiplied indefinitely.

- Prepare culturing media with the addition of malt and coconut milk. Work under sterile conditions.
- Take cuttings from the growing explants and transfer to new microbox or baby jar.

STAGE 3 : ROOTING

- Once there are enough shoots transfer to a rooting medium. Add an extra 100ml of coconut milk with 1/2 teaspoon of malt to the basic media. Ensure that the pH of medium is always between 5 and 6 pH. Adjust pH if necessary with acid e.g. citric acid or bicarbonate soda. Roots will show between 2 - 4 weeks.

STAGE 4 : POTTING

- Once roots have grown to 2cm, transfer to a potting mix. Keep in a warm covered area and out of direct sunlight.

