

RECIPE #01b.

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

Tools and materials

- Micro boxes / test tubes / petri dishes / baby jars
- Cooker / Hotplate
- Pressure Cooker / autoclave- for sterilising
- Pan & spoon
- Air Purifier eg Bionaire and Hepa Filters.
- Sterile transfer chamber eg, perspex box, fishtank, clean room
- Clean sterile surface- tile, sterilised paper ..
- Scalpel / scissors
- Forceps / tweezers
- Burner - use ethanol for burning
- Alcohol - Ethanol at 70%
- Hand / skin disinfectant
- Bleach- ¼ dilution
- Detergent /washing up liquid

Basic media for plant tissue culturing

Culturing media

- Sugar- 100 grams for carbon energy as there is no photosynthesis
- Water soluble fertiliser- 200ml: mix ½ tablespoon all purpose 10:10:10 -N.P.K (nitrogen(N), phosphorus(P) and potassium (K)) in 1L of water.
- Inositol tablets (500 mg)- 1/2 tablet. tissue repair
- Vitamin tablet with thiamine- 1/2 tablet. Any multivitamin tablet may be used. tissue repair

or

- Murashige & Skoog salts (M&S)
- Agar flakes- 100 grams (dependent on density)
- Distilled or rain water - 400ml
- Sterile water- to sterilise boil for 5 mins (+ more for height above sea level) or pressure cook.

Rooting media

the above + variations of the below

- Coconut milk
- Malt- teaspoon
- Citric acid
- Bicarbonate of soda
- Orange juice

These are growth regulators and encourage cell enlargement and division.

- pH indicator tape: 5.0 - 6.0



STAGE 1 : 4 weeks

Culturing

Prep all area's to make sterile as possible.

- a. Mix the basic media over heat- Agar, thiamine, Inositol, sugar until dissolved, add fertiliser.
- b. Pour about 2cm into baby jars and / or Microboxes cover / close and process in a pressure cooker for 15 minutes after pressure is reached (this is when the pressure valve lets steam out). Make sure jars are off the bottom of the pressure cooker.

You will need ½ pt of water for 15mins.

Start timing once the cooker has come up to pressure. Let the cooker come up to room temp before opening.

- c. Sterilise the cabinet by spraying alcohol (70 % ethanol), wipe dry with sterile material or allow to dry. Sterilise all items needed for culturing by dipping / spraying then flaming- scalpel, tweezers, forceps ...
- d. Sterilise water in baby jars or microbox in the pressure cooker.
Seeds & plant material: wash in diluted domestic bleach solution (see below)

1/4 cup bleach
1 drop detergent / washing up liquid
3/4 cup water

Put the plant material in a jar for 10 - 20 mins, agitate frequently then wash in sterile water 2-3 times.

Seeds- put into the same solution in filter paper for a shorter time, try 8 - 11 mins. The ys should go from - black > brown to > straw coloured.

Once washed carefully place one small sterile piece of plant stem / shoot with tweezers into the agar solution in baby jars / microboxes. Make sure not to touch the top. Close lids and keep in a warm light place.

STAGE 2 : 4 weeks

a. Multiplication

This can take place every 4 weeks until there are enough plants.

Multiplication and rooting media

For preparation of the multiplication and rooting media add the same media above and include extra 100ml of coconut milk with 1/2 teaspoon of malt. Ensure that the pH of medium is always between 5 and 6 pH. Adjust pH if necessary with acid e.g. citric acid or base bicarbonate soda.

This stage can be multiplied indefinitely ...

STAGE 3: 2 - 4 weeks

a. Rooting

Once there are enough shoots transfer to a rooting medium with coconut milk and malt. Roots will show between 2 - 4 weeks.

STAGE 4 - 4- 6 weeks

a. Potting Process

Once roots have grown to atleast 2cm, transfer to a potting mix and keep in a covered container in a warm place out of direct sunlight.