TISSUE CULTURE: AN INTRODUCTION TO A USEFUL TECHNIQUE IN ROOT CROP RESEARCH IN PAPUA NEW GUINEA

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INTRODUCTION

Tissue culture is the technique of growing plantlets (small plants) in small bottles or vials (small glass or plastic tubes) (see Figure 1(a)). It is also called in vitro propagation. The plants are not grown in soil but are grown in a 'nutrient medium' which may be a liquid or a solid jelly-like substance. The nutrient medium contains all the minerals, hormones and other chemicals that are necessary for plant tissue to grow and develop.

Plantlets growing in vials are kept in a special, clean growth room, under lights suitable for photosynthesis. Photosynthesis is the process in which green plants use sunlight to make carbon dioxide and water into sugars. The room is kept at a controlled temperature. The rate at which the plants grow can be varied by altering the temperature of the growth room.

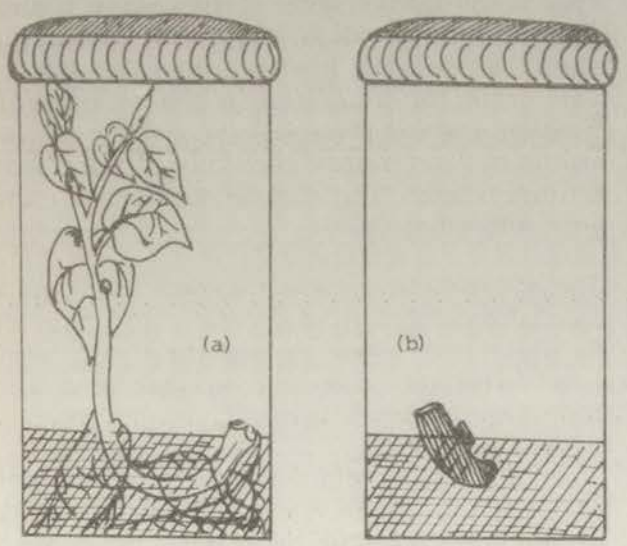

Figure 1. Tissue culture tubes containing (a) a sweet potato plantlet, and (b) a nodal section of sweet potato

USES OF TISSUE CULTURE

There are many reasons for growing plants in tissue culture. Some of the most important are listed below:

1. For the rapid multiplication of planting material. Using tissue culture, a single plant can be cut into very small pieces and each piece can grow into a new whole plant. For sweet potato in tissue culture a single plantlet can be cut into about 10 sections. Each section will grow into a new plantlet. The new plantlets will be ready for sectioning again within 6-8 weeks.

2. So that viruses and some other disease-causing organisms can be eliminated (removed) from planting material. The meristems (tips of the growing shoots), are usually free of viruses. By cutting off the meristems (0.25 - 0.40 mm long) and growing these in tissue culture media, disease-free plants can be established.

3. So that cultivars, especially of root crops, can be exchanged with other countries, with very little risk of introducing new pests and diseases. Plants can easily be transported in small tissue culture tubes.

4. To maintain collections of cultivars of vegetatively propagated crops as a genetic resource for plant breeding. It is sometimes easier to keep the plants in small vials in the laboratory than to plant them in blocks in the field.

5. To germinate seeds that are too small to handle easily in a nursery (e.g. orchid seeds).

6. To develop new cultivars that are resistant to particular diseases. This is done by cultivating single cells of the crop plant in the presence of chemicals related to the disease. Any cells which have mutated (changed their genetic structure) and are resistant to the disease are cultured into complete plantlets.

TISSUE CULTURE MEDIA

The medium is the substance in which the tissue-cultured plants grow. It may be a liquid, or a jelly-like solid. The solid medium is made using a powder called agar which is extracted from sea weed. All the chemicals needed for the tissue to grow and develop are added to the medium.

The best medium to use depends on the type of plants that are being tissue-cultured. Four groups of chemicals are needed in the medium. These are:

1. Mineral nutrients: nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), molybdenum (Mo), boron (B), cobalt (Co), copper (Cu), zinc (Zn), and iodine (I), which are all necessary for meristems or small plant sections to grow and develop in tissue culture.

2. Plant hormones: these are chemicals which control the growth of the plant, especially the development of shoots and roots. The ones usually used are indol-yl acetic acid (I.A.A.) and kinetin.

3. Growth factors: these are other vitamins and chemicals known to help the plant to develop. The most commonly used ones are thiamine hydrochloride, pyridoxine hydrochloride, myo-inositol, glycine and nicotinic acid.

4. An energy source: usually sucrose.

To prepare 1 litre of the solid type of medium, 10 g of agar is heated with 500 ml of water to dissolve it. The chemicals are measured out in the correct amounts and added to the agar solution. The mixture is then made up to 1 litre with water and mixed. It is then quickly poured into the tissue culture tubes (5 ml per tube). The caps must be replaced as soon as the medium has been poured into the tubes. As the mixture cools down it sets to a jelly.

The medium is ideal for plants to grow; but it is also very good for fungi and bacteria to grow. Therefore the tubes containing the media must be sterilised to kill any bacteria, or fungal spores that are inside the tubes. To do this, the tubes are heated to 125 - 130^{o}C for 15-30 minutes in a special oven called an autoclave, and allowed to cool down. They are then ready to receive the small pieces of plant tissue which will grow into complete new plants.

TISSUE CULTURE TECHNIQUES

When the tissue culture vials are ready, a small piece of plant tissue is placed on the surface of the agar (see Figure 1(b)). While this is being done it is necessary to be sure that no bacteria or fungal spores get inside the tube. If they do, they will grow rapidly, and spoil the plant material placed on the agar medium.

The following method is used.

First, the plant material must be sterilised by placing it in a special chemical solution (0.5% calcium hypochlorite) for 10 minutes. This kills the bacteria and fungal spores on the surface of the plant tissue. Next, the chemical is washed off by placing the plant material in sterilised water (water can easily be sterilised in the autoclave). Finally a small piece of the plant material is cut off and placed carefully in the tissue culture tube. The cap is replaced on the tube immediately.

These operations are carried out in a special cabinet called a lamina flow cabinet in which the air is passed through a very fine filter to take out all bacteria and fungal spores (see Figure 2).

When the cap is taken off the tube in order to place the plant material inside, the neck of the tube must be held close to a naked flame (e.g. from a bunsen burner). This kills any bacteria or fungal spores (see Figure 3).

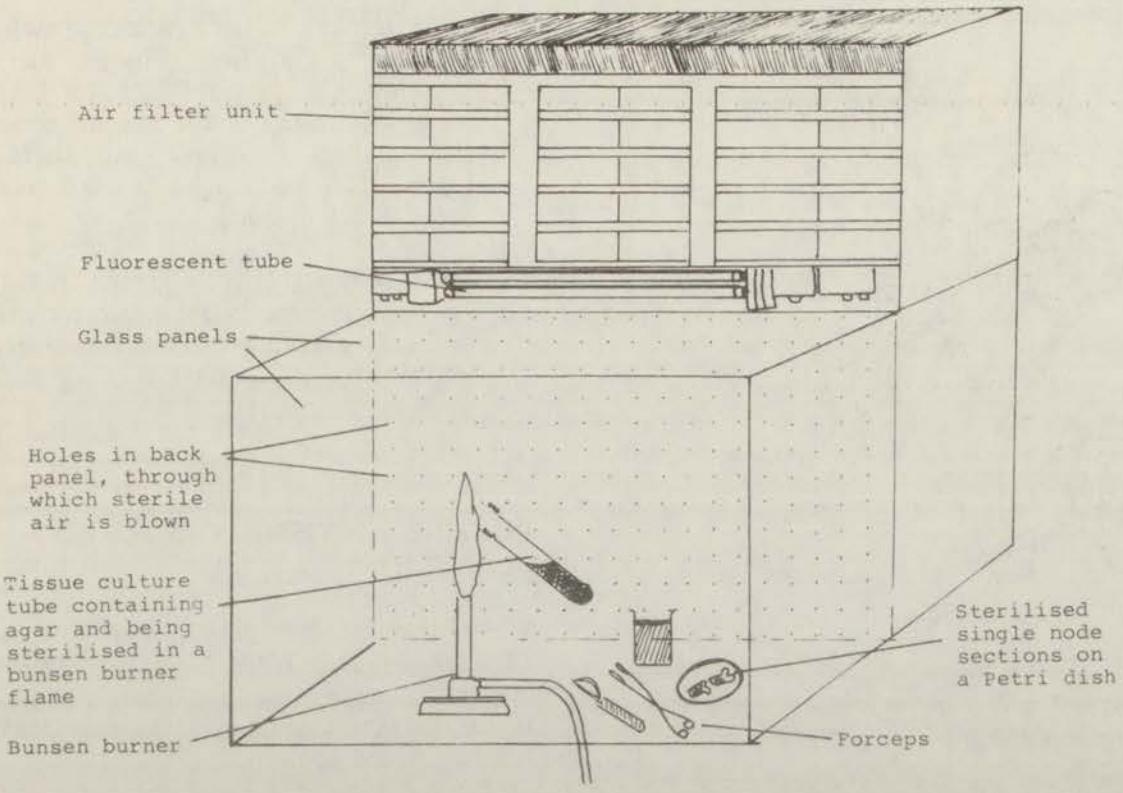

Figure 2. Lamina-flow cabinet used for tissue culture work.

The plant tissue is picked up and placed on the agar in the tissue culture tube using forceps or tweezers. The forceps are first sterilised by placing the tips in alcohol and then in the bunsen burner flame.

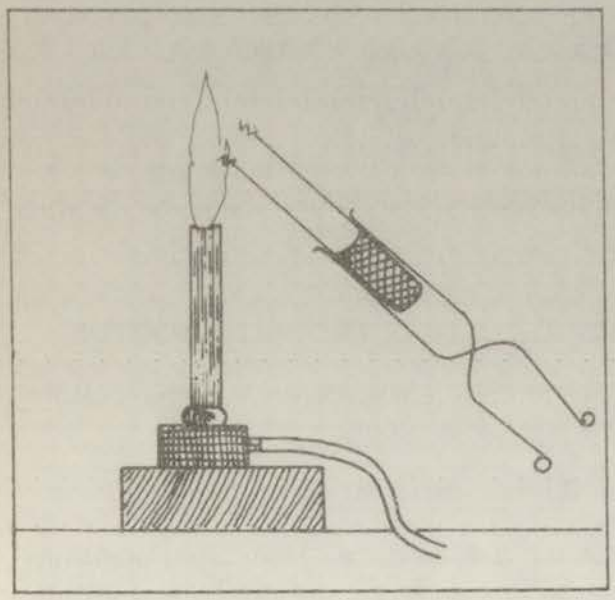

Figure 3. A tissue culture tube being sterilised in the flame of a Bunsen burner.

TYPES OF TISSUE CULTURE MATERIAL USED

This section considers two types of vegetative plant material. The one chosen to be used depends on the purpose of the work. Seed germination techniques and single cell culture are not discussed in this article.

The two types of plant material are:

1. Single node cuttings: These are used for rapid multiplication, and for maintaining cultivars in sterile conditions over long periods. To get single node cuttings, the stem of a small plant, usually from tissue culture, is cut into sections so that each section contains a piece of stem, one leaf and a lateral bud (see Figure 4). Each cutting is placed in a separate tissue culture tube. A knife, scalpel or pair of scissors can be used for cutting the plant material into single nodes. The instrument must first be sterilised as described above for the forceps.

Figure 4. Sweet potato plantlet which has been grown in tissue culture and which has been cut into single node sections for rapid multiplication.

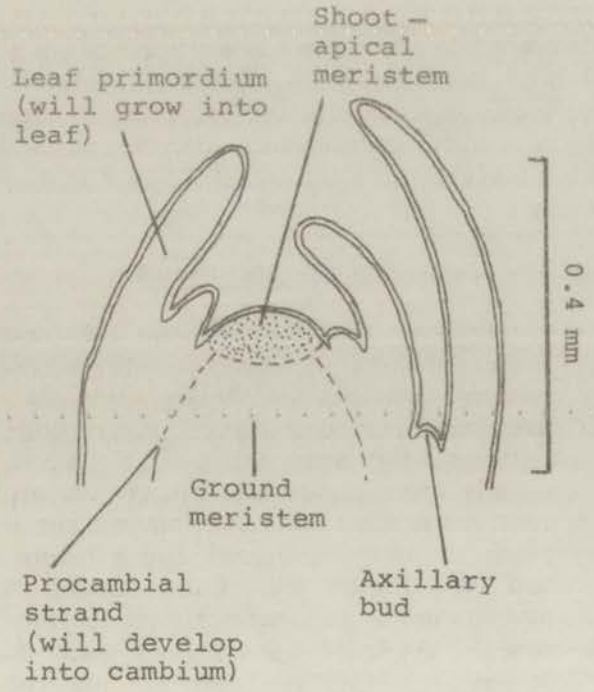

Figure 5. Growing point (apical meristem) of sweet potato vine, as seen under a binocular microscope.

2. Meristems: These are the growing tips of the plant (see Figure 5). The growing tip (about 0.25 to 0.40 mm long) is cut off under sterile conditions and placed on the agar surface to grow. This technique is used to obtain disease-free plantlets.

   When a new plant is grown in this way its disease-free status can be checked by (1) grafting to disease-sensitive indicator plants, or (2) using sensitive pathological tests.

POTTING-ON TISSUE CULTURED PLANTLETS

When the plantlet has grown and almost fills the tissue culture tube, it can either be cut into single node sections and put into tissue culture again, or it can be potted on for continued growth.

For potting-on, soil or potting mix is sterilised and placed in jiffy pots or polybags. The plant to be transferred from tissue culture is removed from its tube, the roots are thoroughly washed to remove the agar - nutrient medium, and the plantlet is carefully planted in the pot and placed in the shade. The potting mix must be kept very moist for the first 2-3 days otherwise the plant will wilt. For the first 2-4 days placing a polybag over the plant helps to prevent too much moisture loss and plant stress after potting on.

When the plants are big enough they can be transferred to larger polybags or to the field.

TISSUE CULTURE TROUBLE-SHOOTING

Some of the main problems in tissue culture work are listed below:

1. Contamination of apical meristems

Growing tips collected from field plantings are often difficult to surface sterilise because the material has been exposed to soil splash and bacteria and fungal spores carried by wind.

It has been found that much better results can be achieved if tubers are sprouted in the laboratory. These sprouts can then be used as a source of meristem material.

2. Callousing of apical meristems

Meristems placed on the culture medium often form large callouses (lumps of tissue) but shoots and roots do not differentiate.

It has been found that reducing the amount of cytokinin (kinetin) in the medium to a low level, or completely eliminating it, results in a greatly improved growth of meristems.

3. Stunted growth

Sometimes plants growing in tissue culture tubes have a 'spiky' appearance. The stems are relatively thick and very few leaves develop.

This is usually caused by a shortage of oxygen in the tubes for the plants to respire (breathe). Loosening the caps of the tubes to allow air to enter may solve this problem, but the caps should not be removed completely. Even just loosening the cap may increase the risk of contamination.

4. Etiolation (long thin plants)

Sometimes plants grow very thin and spindly in the tubes. This is caused by not enough light, or by havivg the temperature too high in the growth room.

CURRENT USES OF TISSUE CULTURE WITHIN DPI

Recently, DPI has used tissue culture mainly to receive new cultivars of Irish potato and sweet potato from overseas. Thirty-four new cultivars of sweet potato have been received from the Asian Vegetable Research and Development Centre in Taiwan, together with 21 cultivars from the UNDP Project in Suva. Thirty-two new cultivars of Irish potato were sent from the International Potato Centres in Peru and the Philippines in tissue culture form.

FURTHER INFORMATION

For further information about the methods and uses of tissue culture, you can contact the author:

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(Illustrations: Pawal Andy (Figs 1 - 4)