PACIFIC REGIONAL AGRICULTURAL PROGRAMME

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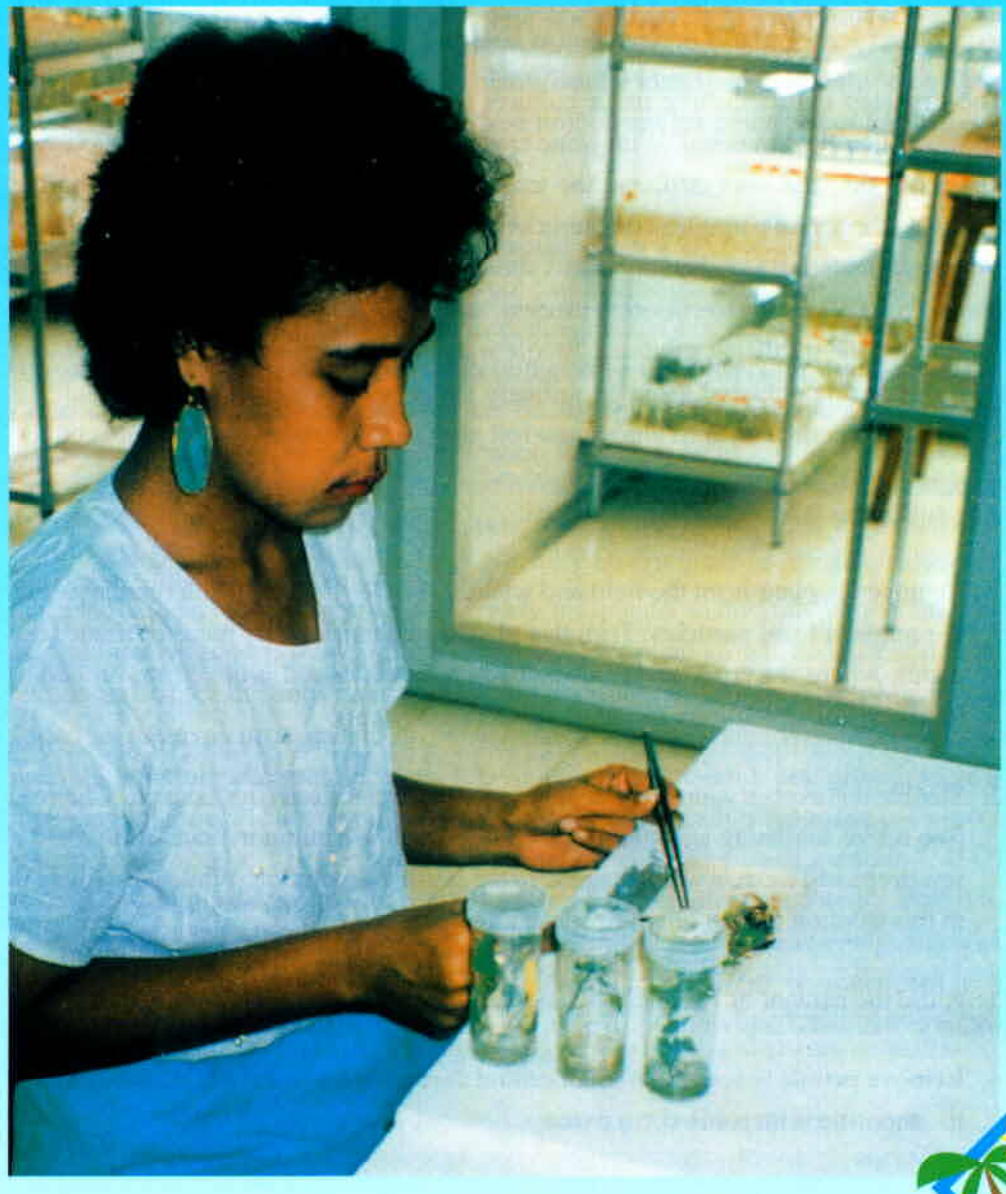
TISSUE CULTURE OF TARO

(COLOCASIA ESCULENTA VAR. ESCULENTA)



PRAP

INTRODUCTION

The PRAP/IRETA Tissue Culture Unit maintains, multiplies and distributes a number of crops; these include taro, sweet potato, cassava, banana, vanilla and yams. This leaflet describes the tissue culture of *Colocasia esculenta* var. *esculenta*, (taro, dalo, dasheen). The techniques involved in the tissue culture of all crops are basically the same, but each crop has its special requirements. This leaflet also aims to show that these techniques are relatively simple, and that complex equipment is not essential for success.

REMOVAL OF PATHOGENS FROM FIELD MATERIAL

The first step in establishing tissue cultures from field material involves the cleaning-up of that field material. With some crops this first step can be difficult, however this is not the case with taro, and the technique described below is effective in the removal of external pathogens (bacteria and fungi). When establishing tissue cultures from field material, only healthy plants should be used. Very simple equipment is required to carry out the sterilisation process, and beakers or old glass jars are suitable for holding the sterilising solutions. Sterile water and sterile instruments (forceps and scalpels) can be prepared using an ordinary pressure cooker. Instruments should be wrapped in a double layer of aluminium foil prior to sterilisation in a pressure cooker.

The following procedure should be used:

1. Remove the plant from the field and where possible rinse well with running water to remove all soil particles. Trim this plant down to a smaller piece of tissue (explant), where the corm measures 5–10 mm in width and up to 5 mm in depth, together with 15–30 mm of petiole base tissue (stem).

2. Sterilise this explant with a 10% solution of commercial bleach, for example, Chlorox, (the active sterilising agent in commercial bleach is sodium hypochlorite). Add a few drops of Tween, a wetting agent, to the sterilising solution. While the explant is in this solution for ten minutes, shake a few times.

3. Rinse the explant three times with sterile, distilled water.

4. Remove petiole bases until a small central core of leaves is reached; this explant is the shoot-tip with some corm tissue.

5. Repeat the sterilisation process but using 5% Chlorox plus a few drops of the wetting agent, Tween, for 5 minutes.

6. Rinse the explant four times with sterile, distilled water.

The final size of the explant, (step 4), depends whether adequate equipment, such as a dissecting microscope, is available. Small explants, such as meristems (the growing point of the plant), are only excised if virus-free plants are required; these explants are very small, measuring approximately 0.1 mm in diameter and 0.2–0.4 mm in length. For the establishment of tissue cultures, an explant of a relatively large size is acceptable. Excision down to the meristem is often necessary if persistent bacterial contamination is suspected. If endogenous bacterial contamination is a problem, then procedures can be followed so that 'dirty' explants can be identified, and only 'clean' explants are established in tissue culture. These procedures are described in *PRAP Leaflet*, no 4, 'Endogenous Contaminants and Tissue Culture'.

Ideally, all steps from step (4) should be carried out in a laminar airflow cabinet, i.e. under sterile conditions, but with care shoot-tips can be excised in an ordinary room with no air conditioning with minimum contamination levels. What is essential in this situation is speed, clean instruments (either soak in Chlorox, or use alcohol and flame in between operations) and the use of 70% alcohol for swabbing hands and surfaces. Using this technique, contamination levels can be as low as 10%.

PREPARATION OF THE CULTURE MEDIUM

Once the explant has been prepared it is ready for transfer to the culture medium. The culture medium must provide all factors essential for plant growth and development, and so must include water, macronutrients, micronutrients and sugars. Sugars are added as the explant is unable to photosynthesise effectively. With some culture medium other organic substances such as vitamins, amino-acids and growth regulators, are also added. The taro multiplication process is basically simple, but consists of a four-stage process. For germplasm maintenance either the basal medium (MSO) without any additions can be used, or the same medium supplemented with 1.0 mg/l 6-benzylaminopurine and 0.3 mg/l α-naphthaleneacetic acid. The basic composition of macro- and micronutrients, that is, the basal medium MSO can be purchased as a ready-mixed powder, but the medium can be made using individual chemical components (stock solutions).

A magnetic stirrer is required to ensure all medium components are well-mixed, and a pH meter to achieve the correct pH (5.5–5.8). Once the medium is prepared a gelling agent (agar or Gelrite) is added to solidify the medium, which is then poured into the culture containers (anything from test tubes to glass jars). The containers and media are then sterilised, and this can be done in a pressure cooker. Effective sterilisation requires 121°C and 1.06 bar pressure for 15 minutes, or 20 minutes for larger volumes.

The four stage process required for optimum multiplication of taro in tissue culture is as follows:

Stage 1: MSO + 0.025/0.05 mg/l thidiazuron (TDZ)

Stage 2: MSO + 0.8 mg/l BAP

Stage 3: MSO + 0.005 mg/l TDZ

Stage 4: MSO (liquid stage)

The basal medium (MSO) is Murashige and Skoog Plant Salts (1962) supplemented with 100 mg/l myo-inositol, 0.4 mg/l thiamine HCl, 30 g/l sucrose and 1.75 g/l Gelrite or 8 g/l agar. The concentration of TDZ used depends on the sensitivity of the plant; with some varieties of taro 0.05 mg/l TDZ can be phytotoxic. The duration of each culture stage is three to four weeks. On subculturing it is important not to trim down the plantlet too much as this will tend to remove the shoot buds that have been initiated with the use of TDZ. The roots must be trimmed without any damage to the corm, and the height of the plantlet should be reduced to approximately 50% so that apical dominance is inhibited. For optimum development of the plantlets the final stage should be liquid culture, and preferably shaken.

GROWTH OF THE TISSUE CULTURE PLANTLET

The excised shoot-tip is partially inserted into the medium and then the container plus the explant is placed where light and temperature conditions are relatively controlled. Obviously extreme fluctuations in both have to be avoided. Growth rooms can be very sophisticated but plant tissue cultures can be grown in rooms where the only source of light is good, natural light. The temperature range for optimum growth of most tropical plants is 25°C to 28°C.

Once stage 4 is reached, depending on the variety used, it will take approximately four to eight weeks for a shoot-tip to develop into a plant that can be transferred to the soil. As stated this process can be speeded up through the use of liquid culture. Liquid culture is more effective if the culture containers are gently shaken to improve aeration and absorption of the nutrients. The equipment for use with liquid cultures can be expensive, but a simple and adequate piece of equipment can be made using an old motor. Although shaking of the cultures promotes the development of the plants, it is not essential.

WHEN IS THE CORRECT TIME TO SUBCULTURE?

As previously stated subculturing should take place every three to four weeks, and care should be taken in trimming down the plantlet. If too much tissue is removed around the corm then this will also remove initiated shoot buds. When dividing up suckers, only those of a reasonable size, (>5 mm) should be removed and cultured individually. The use of TDZ can often result in the production of very small suckers that are difficult to isolate as individual plantlets. In this case, suckers should be subcultured as clumps, and divided later. Hyperhydricity and fasciation are problems that have been observed when TDZ is used in a multiplication system (Huetteman and Preece, 1993). Shoot fasciation is described as the fusing of multiple shoots to form a single stem; fasciation has been described in taro as 'shoots comprised of enlarged and flattened petioles with small protruding leaf-like structures giving the appearance of several shoots fused together' (Palupe, 1997).

If the tissue cultures are being maintained as part of the germplasm collection, then subculturing can wait until either the plant is 'too big' for the container, or the medium is used up. Care must be taken not to delay subculturing for too long as the culture can get infected, and the plant weak. Most plants will require subculturing after four months.

TRANSFERRAL OF THE TISSUE CULTURE PLANTLET FROM THE TUBE TO THE SOIL

Transferring the tissue culture plantlets to the soil has been described in *PRAP Leaflet* no. 5. This stage is very important, as losses here mean that all that earlier work has been a waste of time.

THE PROBLEM OF ENDOGENOUS CONTAMINANTS

Tissue cultures of taro can suffer from endogenous bacteria infection. Some cultures were sent from this laboratory to the Central Science Laboratory, UK, where it was found that eight of the ten cultures examined contained *Methylobacterium mesophilicum* and other cultures contained *Pseudomonas fluorescens* complex and *P. syringae* complex. These were not as abundant as the pink *Methylobacterium* sp. Cultures infected with a low level of bacteria can be cleaned effectively with a solution of 20% Chlorox plus a few drops of the wetting agent, Tween, for 20 minutes. This sterilisation treatment has to be combined with reducing the size of the explant, that is, removing leaf and corm tissue. Care must be taken not to make the explant too small as size can affect the recovery from the sterilisation process. Continuous shaking of the explant in the Chlorox solution is often necessary for effective sterilisation. Similarly, carrying out the sterilisation with Chlorox under vacuum results in the loss of air bubbles and the sterilisation process is more efficient. When the level of contamination is high, treatment with antibiotics is necessary; to date rifampicin has been the most effective in removing bacterial contamination from taro explants.

THE MULTIPLICATION RATE OF TARO IN TISSUE CULTURE

The taro multiplication that was in use in this laboratory did not optimise suckering *in vitro*; the number of suckers produced varied according to the variety. In using TDZ this varietal influence has been reduced, as suckering has been significantly increased. With the new system it is now possible to produce 11.4 million suckers per annum from one sucker, depending on the variety in use.

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