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PRACTICAL MANUALS FOR HANDLING CROP GERMPLASM *IN VITRO* 1.

MERISTEM-TIP CULTURE AND VIRUS INDEXING OF SWEET POTATOES

by

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FOREWORD

In vitro methods are expected to assume an increasingly important role in the conservation of plant genetic resources, particularly for clonally-propagated samples, or for species which, for various reasons are difficult to conserve as seed. Broadly speaking, two groups of crop plants are involved: the root and tuber crops, many of which are tropical, such as cassava, yam and sweet potato; many tropical tree fruits and several industrial crops such as rubber and cocoa. This booklet, the first in a series dealing with specific crop plants, collates the available technical data on *in vitro* methodology appropriate to sweet potato and also addresses the problem of obtaining virus-free germplasm for distribution and conservation, an area in which tissue culture also has a vital role to play.

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INTRODUCTION

In August 1980, an IBPGR Working Group on the Genetic Resources of Sweet Potato met in Charleston, South Carolina, USA, to review the status of sweet potato germplasm and discuss possible steps to aid its conservation (IBPGR, 1981). The Working Group concluded that the establishment of an international repository would greatly enhance the potential of the crop. Justifications for an international germplasm system were given as follows:

- Sweet potato is an important crop ranking seventh in terms of world-wide production.

- Based on calorie yields, nutritional value, adaptability, versatility, its tropical origin and vegetative reproduction, the sweet potato could, in the future, make a greater contribution to world agriculture than it does at present.

- The risk of losing significant amounts of germplasm is already high and is expected to increase.

- The establishment of an international repository for sweet potato germplasm is expected to expedite research and germplasm exchange.

The Working Group recommended the organization of a world-wide system of germplasm collections with a limited number of field genebanks. In order to make an international germplasm system for sweet potato feasible, two major problems had to be addressed. The first was the need for quarantine; only pathogen-free material must be transported internationally. The second problem stems from the fact that the sweet potato is vegetatively propagated. In order to maintain the genotypic integrity of improved genotypes, they must be propagated clonally and maintained in vegetative form at the repositories. Clonal propagation and storage of sweet potato by traditional means is time- and space consuming and costly. Meristem-tip culture and *in vitro* maintenance of plantlets can reduce these inputs.

This manual is designed to serve as an information source for methods of meristem-tip culture, *in vitro* propagation and virus indexing of sweet potatoes. An effort has been made to keep the methods simple so they can be adapted to most laboratory situations.

CULTURE PROCEDURES

This section details the materials and methods involved in the meristem-tip culture of sweet potato and the subsequent *in vitro* clonal propagation of plantlets derived from meristem-tips.

Materials and Equipment

Plant Material

Either sprouted storage roots or rooted shoots from established plants can be used as sources of buds for meristem-tip culture. Glasshouse or growth chamber facilities are necessary for the maintenance of parent material. Clean, sterile sand provides a good sprouting medium for roots and the buds produced are relatively free of micro-organisms.

Culture Media

The basic nutrient solution of Murashige and Skoog (1962) is very suitable for sweet potato meristem culture (Table 1). M&S salts can be purchased in premeasured packages from many tissue culture suppliers. Table 2 lists additional compounds used in the media at various stages of culture.

Media Preparation Area

Any suitably equipped laboratory space can serve as a media preparation area. Essential equipment includes a pH meter, an autoclave or pressure cooker, a hotplate (or steam bath), a metering device to measure out small amounts of hot medium (a spring-loaded syringe will suffice) and a refrigerator in which to store prepared media. Glassware should include an assortment of beakers, Erlenmeyer flasks, graduated cylinders, volumetric flasks, pipettes and storage bottles. Vials (25 mm x 100 mm) with polyethylene caps are very suitable culture tubes, although many types of container can be used successfully, including discarded medical vials, as long as they can withstand autoclaving.

A source of distilled water is essential and a source of deionized water is very useful. Apparently unidentifiable problems with meristem culture, particularly the unrepeatability of results, may often be traceable to impurities in the water used.

Culture Preparation Area

The maintenance of aseptic conditions is the most important factor to consider when setting up a culture preparation area. Ideally, the inoculation area should be under a laminar air-flow hood. However, a small covered inoculation hood or even a small isolated room in which air movement can be kept to a minimum, and which can be kept scrupulously clean, may be adequate.

Table 1. Composition of Murashige and Skoog (1962) basic salt solution (M&S)

Major Salts		Minor Salts	
Salt	mg/l	Salt	mg/l
NH_4NO_3	1,650	H_3BO_3	6.2
KNO_3	1,900	$MnSO_4.4H_2O$	22.3
$CaCl_2.2H_2O$	440	$ZnSO_4.4H_2O$	8.6
$MgSO_4.7H_2O$	370	KI	0.83
KH_2PO_4	170	$Na_2MoO_4.2H_2O$	0.25
Na_2EDTA	37.3	$CuSO_4.5H_2O$	0.025
$FeSO_4.7H_2O$	27.8	$CoCl_2.6H_2O$	0.025

Table 2. Organic additives in sweet potato meristem culture media

Component	mg/l
Sucrose	50,000
Thiamine	2
Myo-inositol	100
Na_2EDTA 1/	37.3
α - napthalene acetic acid (NAA)	0, 0.03
6 - benzyl-amino purine (BAP)	0, 0.30
Agar 2/	7,000

1/ EDTA in excess of the concentration found in M&S salts is added to chelate all free iron. This prevents iron precipitation of phosphates with consequent fluctuations in pH.

2/ Agar should be of high quality and preferably of a grade prepared specifically for tissue culture work.

Equipment and supplies needed for the culture preparation area (Fig. 1) include ethanol, commercial bleach (containing sodium hypochlorite), an alcohol lamp, a dissecting microscope, and an assortment of dissecting instruments. The latter should include surgical scalpels with a supply of removable blades (straight, sharp-tipped), fine-tipped forceps, and fine-tipped dissecting needles. Some researchers have also reported that a lens knife or micro-scalpel made from a piece of razor blade glued to the end of a small wooden handle makes an effective tool for the removal of meristems (Mori, 1971; Roca, 1980).

Incubation Area

Sweet potatoes can be regenerated successfully under a relatively wide range of environmental conditions, and consequently a simple incubation area can serve quite well. Ideally, the area should be temperature-controlled. A constant temperature of 27°C will give rapid regeneration without excessive dehydration of the culture medium. However, if the temperature does not exceed a maximum of 34°C or a minimum of 22°C, complete control is not absolutely necessary. Banks of fluorescent lights should provide illumination in the range 1,500-1,600 lux. Optimum lighting conditions have not been established but fluorescent lighting of 4,000 lux on a light/dark schedule of 16/8 hours is very satisfactory for regeneration. Higher light intensities (above 4,000 lux) induce more rapid regeneration. Supplementary incandescent lighting is not essential but does appear to cause more rapid expansion of shoots once growth commences.

Up to 700 cultures can be maintained under 1 m^2 of light banks.

Plant Establishment Area

Conventional glasshouses can be used to establish regenerated plantlets. Some facility should be made for partial shading.

Planting containers used should have a transparent plastic cover to retain moisture whilst admitting light. Horticultural propagators can be purchased or substitutes inexpensively constructed by using a large clear or opaque plastic drinking cup for a pot and a second clear drinking cup for a cover (Fig. 2). The pot should have a hole in the bottom for drainage, and the covering cup should have a hole for gas exchange. A medium diameter cork borer can be used for cutting holes in the cups.

A 1:1 sand:soil (volume:volume) mixture is an excellent medium for the *in vivo* establishment of plantlets. An all-purpose water soluble fertilizer with trace elements is useful for maintaining a rapid rate of growth.

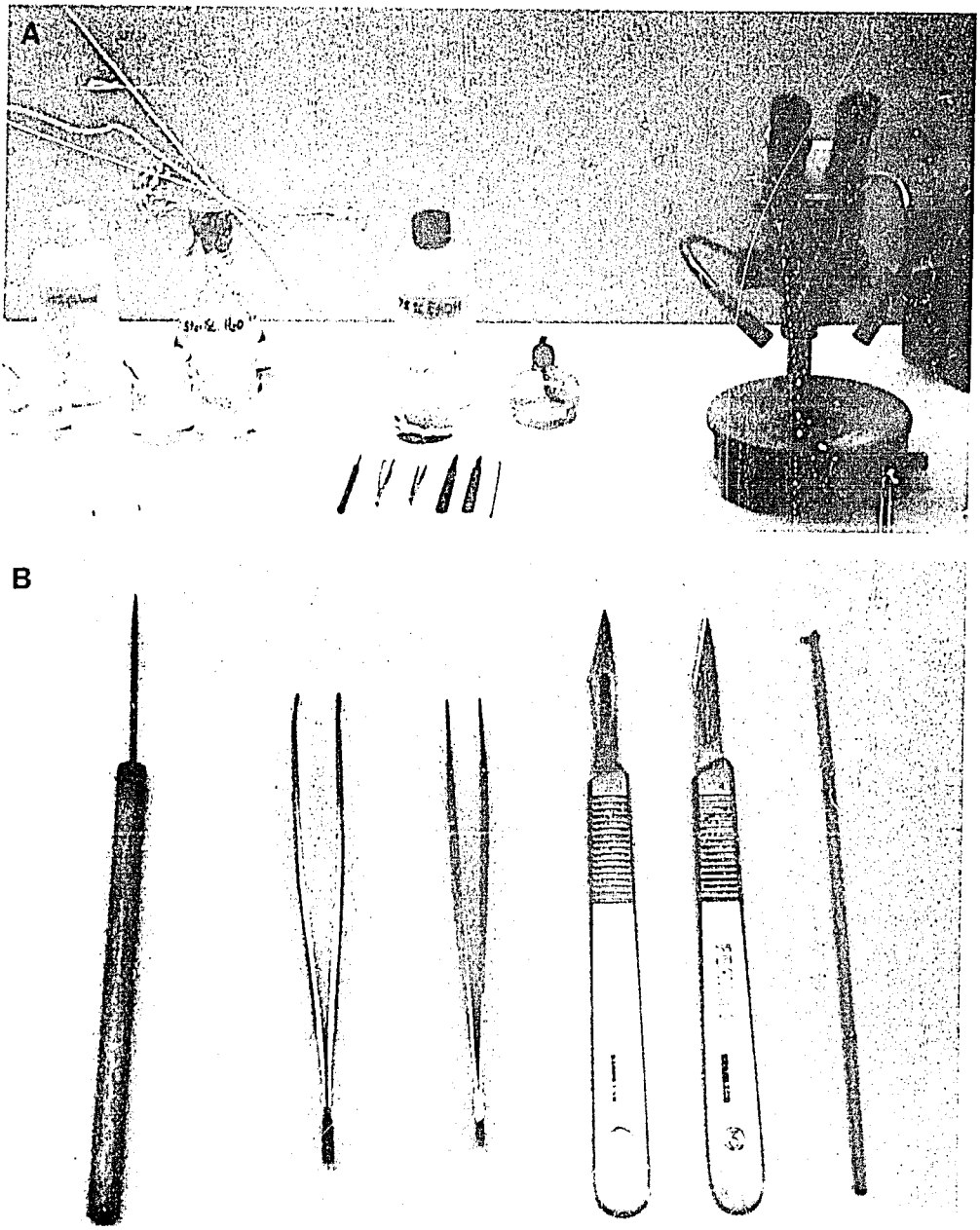

Figure 1. Equipment and supplies needed for the culture preparation area: (A) From left to right: 10% bleach solution, sterile water, ethanol, dissecting tools, alcohol lamp, Petri dishes, and dissecting microscope with lamp. (B) Close-up view of dissecting instruments. From left to right: mounted needle, curved and straight, fine-tipped forceps, scalpels, microscalpel.

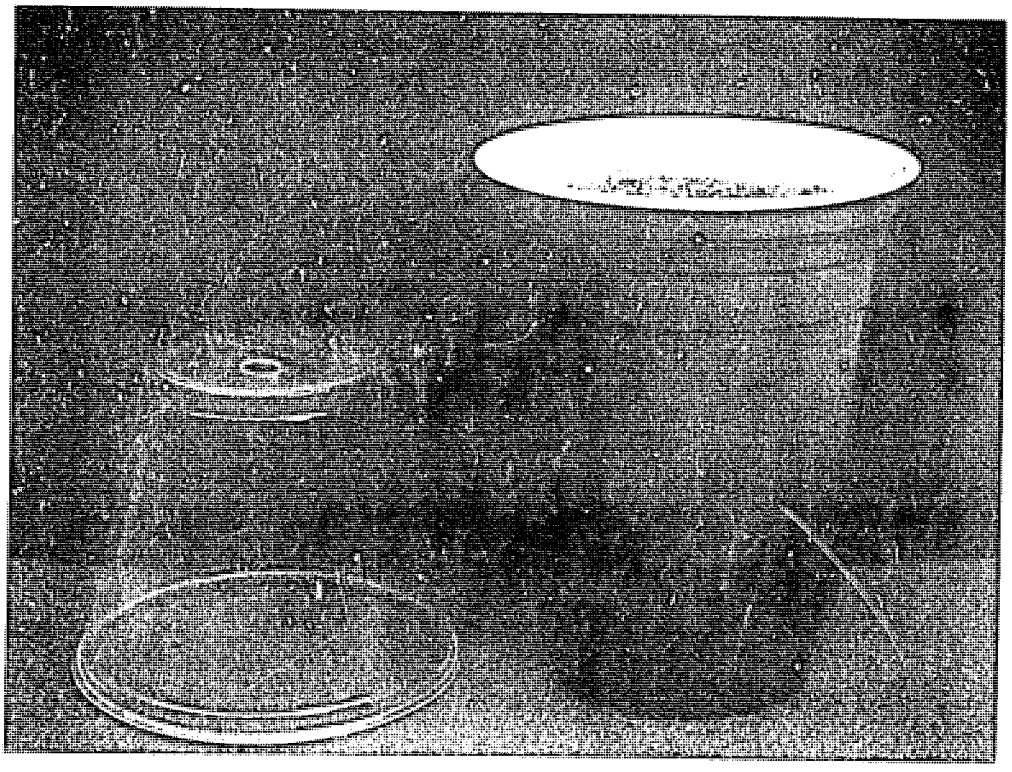

Figure 2. Inexpensive container for establishing regenerated plantlets in soil. Both pot and cover are made from plastic drinking cups.

Methodology

Maintaining Plant Material

Two methods for maintaining a supply of plant material for meristem culture are as follows:

1. Plant healthy sweet potato roots in clean, sterile sand in a glasshouse (stem cuttings from growing plants can also be used; Fig. 3A).

2. Place healthy roots on top of a container of water with about one-third of the root immersed. Ordinary glass bottling jars make suitable containers. Three toothpicks inserted partially into the root, and radiating from the longitudinal centre, will prevent the root from falling into the jar (Fig. 3B).

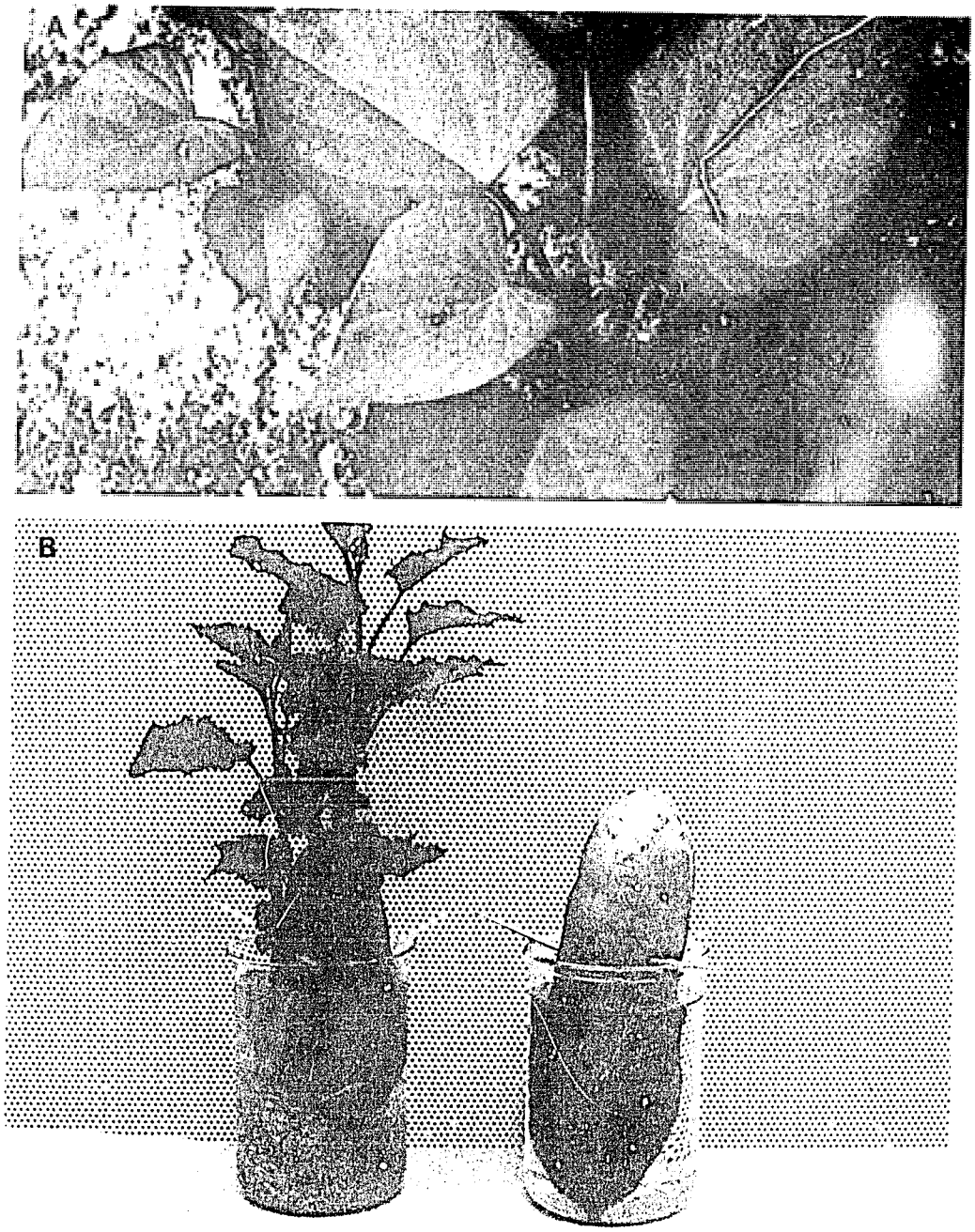

Figure 3. Methods for maintaining parent stock plants for sweet potato meristem culture: (A) Plants growing from roots in clean sterile sand (B) Plants growing from roots placed in water in a growth chamber. Toothpicks inserted into the roots prevent them from falling into the containers of water.

Each method has its advantages and disadvantages. Culture in sand can provide a steady supply of shoots from which meristems may be dissected for an indefinite period of time. However, control of insects and contaminating micro-organisms is difficult. Water culture will supply meristems for a short period of time (2-3 months), but the plants are kept in a cleaner, more controlled environment.

When sprouts from the roots reach a height of 10-20 cm they are ready to be harvested for meristem-tip culture. The time from planting to harvesting is usually 2-4 weeks.

Media Preparation

Precise media preparation is critical to the success of meristem-tip culture. The media composition and preparation techniques described here have been used in our laboratory to regenerate plantlets from meristems of over 40 sweet potato genotypes originating from a wide genetic base.

Two media are recommended for use at various stages of meristem regeneration and clonal propagation (see below and Tables 1 - 4).

Stock Solutions for Use in Media Preparation

Media are most easily and rapidly prepared by diluting concentrated stock solutions. Table 3 gives the composition of the stock solutions and the amount of each stock to be used for preparing media. If premeasured M&S salts are available, only stock solutions 6-9 are needed. Otherwise, all of the stock solutions except 6 will be needed. The use of premeasured M&S salts saves a great deal of time and effort and is well worth the extra cost.

All of the stock solutions have a limited storage life and must be discarded periodically and freshly prepared. The storage times and temperatures suggested for each stock solution are given in Table 4. To avoid wastage, the quantities of each solution prepared should take into account the storage life and amount used per litre of medium.

General Media Preparation Instructions

Instructions for preparing 1 litre of finished Excision and Induction or Regeneration Medium (see Table 3) are as follows (see: de Fossard, 1976):

1. Pour approximately 900 ml of distilled, deionized water into a 2-litre flask. Do not heat.
2. Add the M&S salt mixture. If using premeasured salts, be sure to rinse out the package to remove all traces of residual powder. If using stock solutions, add 20 ml of

Table 3. Composition and amount of stock solutions used in preparing media for sweet potato meristem culture 1/

Stock solution	Ingredient	Amount (mg)	Amount of Stock Solution in medium (ml)	
			Excision and Induction	Regeneration
1 2/,3/	NH_4NO_3	82,500	20	20
	KNO_3	95,000		
	$MgSO_4.7H_2O$	18,500		
	KH_2PO_4	8,500		
2 3/,4/	H_3BO_3	620	1	1
	$MnSO_4.4H_2O$	2,176		
	$ZnSO_4.7H_2O$	860		
	$Na_2MoO_4.2H_2O$	25		
	$CuSO_4.5H_2O$	2.5		
	$CoCl_2.6H_2O$	2.5		
3 3/,4/	KI	83	1	1
4 3/,4/	$CaCl_2.2H_2O$	14,500	3	3
5 3/,5/	Na_2EDTA	2,984	5	5
	$FeSO_4.7H_2O$	1,114		
6 4/,6/	Na_2EDTA	3,730	1	1
7 4/	Thiamine	200	1	1
	Myo-inositol	10,000		
8 7/	BAP	100	3	0
9 8/	NAA	10	3	0

1/ Adapted from Roca (1980).

2/ Dissolve in distilled water. Bring the final volume to 1,000 ml.

3/ This stock solution not needed if premeasured M&S salts are used.

4/ Dissolve in distilled water. Bring the final volume to 100 ml.

5/ Dissolve each constituent in separate containers of distilled water. Heat slightly, mix the 2 solutions and bring the final volume to 200 ml.

6/ This stock solution not needed if stock solutions 1 through 5 are used rather than premeasured M&S salts.

7/ Dissolve BAP in 10 ml 0.5 N HCl. Heat slightly if necessary. Bring the final volume to 1,000 ml with distilled water.

8/ Dissolve NAA in 3 ml 0.5 N KOH. Bring the final volume to 1,000 ml with distilled water.

Solution 1 (macro-nutrients), 1 ml of Solution 2 (micro-nutrients), 1 ml of Solution 3 (KI), 3 ml of Solution 4 ($CaCl.2H_2O$), and 5 ml of Solution 5 (Na_2EDTA and $FeSO_4 7H_2O$).

3. Add 50 g of sucrose.
4. If using premixed M&S salts, add 1 ml of Solution 6 (Na_2EDTA).
5. Add 1 ml of Solution 7 (myo-inositol, thiamine).
6. For the Excision and Induction Medium, add growth regulator stock solutions: 3 ml of Solution 8 (BAP) and 3 ml of Solution 9 (NAA). (No growth regulators are included in Regeneration Medium.)
7. When all components are dissolved, add 7 g of 'tissue culture grade' agar.
8. While the solution is still at room temperature, adjust the pH of the medium to 5.2 using either 0.1 N KOH or 0.1 N HCl.
9. Heat and stir continuously until the agar is dissolved. The solution will become clear. Do not boil.
10. Dispense 10 ml of hot medium into each culture tube. Cap the tubes and autoclave for 15 minutes at 15 p.s.i. (1.06 kg/cm^2 = 121°C).
11. Slant the tubes at an angle of 30°; allow them to cool and the agar to solidify. If the tubes are not to be used immediately, refrigerate them.

Table 4. Storage life and temperatures of media stock solutions detailed in Table 3

Stock	Storage Life (months)	Storage Temperature (°C)
1	6	4
2 1/	6	-20
3	6	4
4	6	4
5	6	4
6	6	4
7 1/	2	-20
8 1/	1	4
9 1/	1	4

1/ Alternatively, stock solutions 2 and 7 can be kept at 4°C for a shorter period of time, and solutions 8 and 9 can be kept at -20°C for a longer period of time (Roca, 1980).

Isolation of Meristem-tips

The procedure for isolation of sterile, healthy meristems is divided into 2 steps: (1) preparation and disinfection of donor plant material, and (2) excision.

Disinfection

Because plant material carries many superficial organisms, a surface sterilization procedure must be used to reduce the number of micro-organisms on the sweet potato stems. It reduces the risk of transferring contaminants into the culture tube along with the meristem-tip. The procedure detailed below and shown in Figs. 4-8 has been found to reduce contamination to less than 5% on meristem-tips taken from glasshouse grown plant material.

1. Select healthy, rapidly growing parent plants from which to remove buds (Fig. 4).
2. Remove the terminal bud along with 2-3 cm of the stem (Fig. 5). Be careful not to allow the stems to wilt after removal.
3. Remove all but the smallest leaves from the stems (Fig. 6). Subsequent steps should be conducted using sterile containers and utensils.
4. Move the stems to the sterile working area and place them in a 10% solution of commercial bleach (0.525% sodium hypochlorite) for 15 minutes (Fig. 7). Add a few drops of 'Tween 20' to the bleach solution to reduce the surface tension of the water and allow better penetration of the sterilant. Remove the stems from the bleach solution and rinse for 2 minutes in sterile water. Repeat the rinse a second time.
5. Place the stems in a sterile Petri dish lined with filter paper to draw off excess water and keep the stems moist (Fig. 8). At this point the stems are ready for excision of the meristem-tip.

Meristem-tip Excision

Excision of the meristem-tip ('apical dome') requires a great deal of patience and skill; it is recognized that every person will develop their own technique. Some aspects of the excision process are critical. Among these are the maintenance of sterile conditions, including the prevention of virus transmission on the excision tools, and the removal of the apical dome without excessive damage in order to ensure its survival.

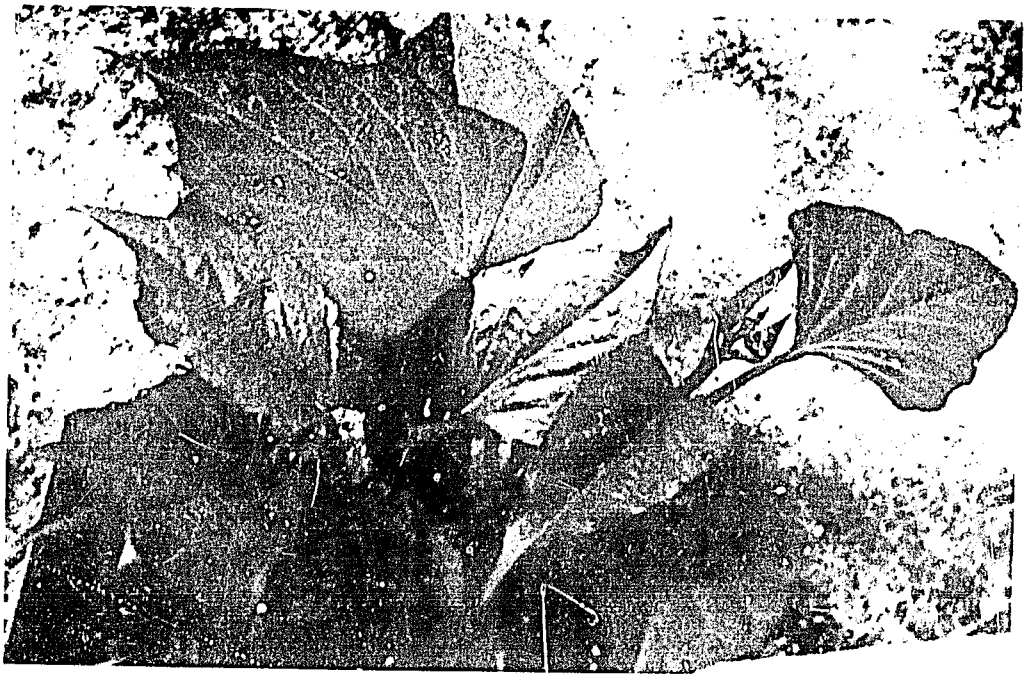

Figure 4. Explant preparation and disinfection procedures - Step 1: Identify healthy, rapidly growing plants to use as a source of meristems.

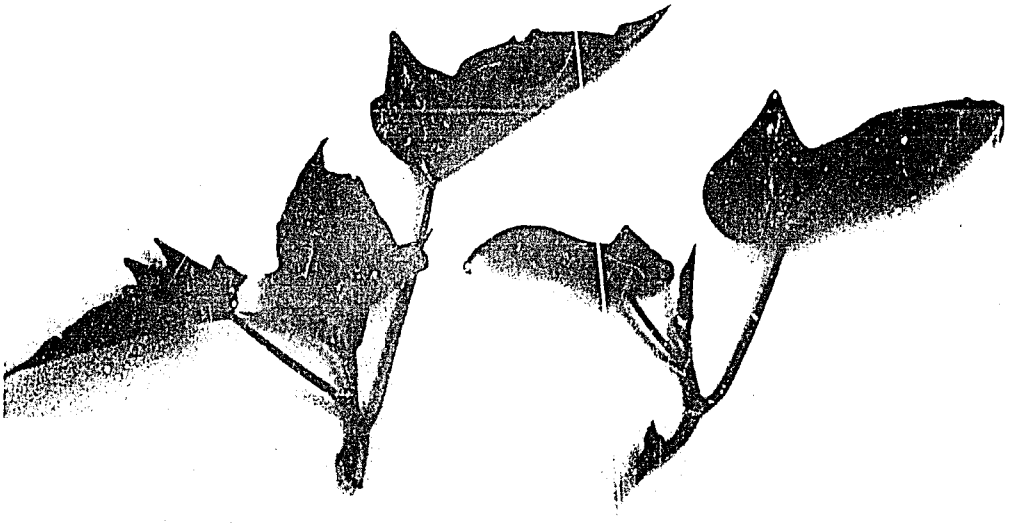

Figure 5. Explant preparation and disinfection procedures - Step 2: remove the apical 2-3 cm from stems of healthy sweet potato plants.

Figure 6. Explant preparation and disinfection procedures - Step 3: remove all but the very smallest leaves.

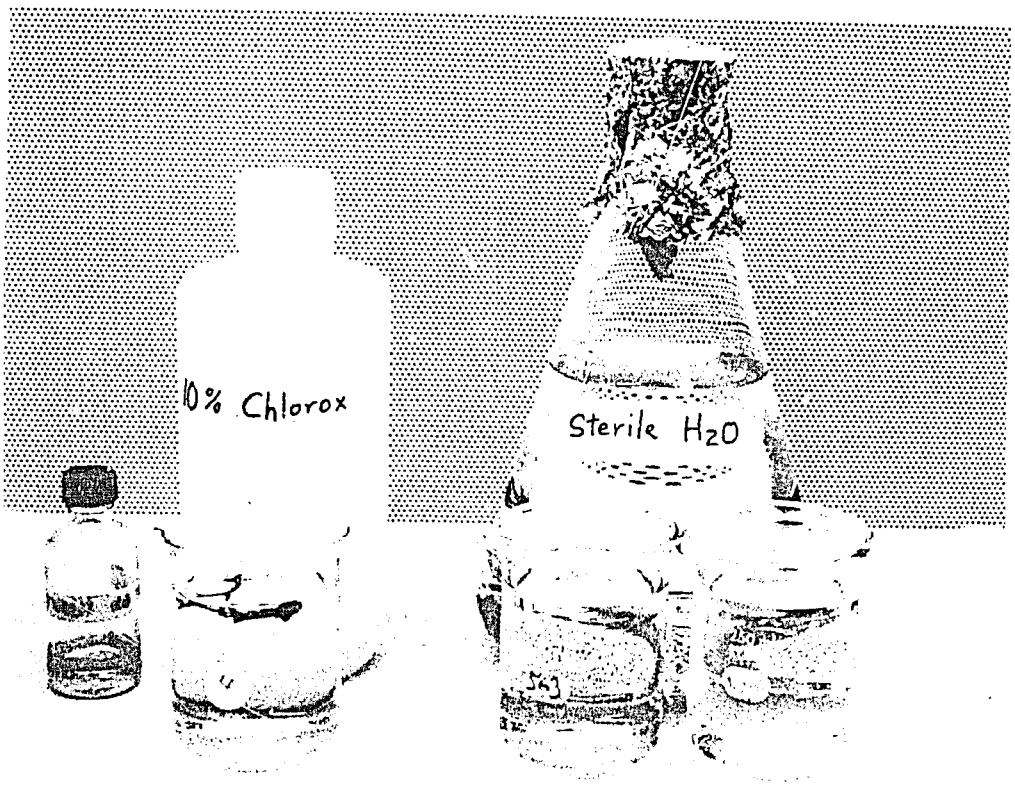

Figure 7. Explant preparation and disinfection procedures - Step 4: immerse the stems in a solution of 10% commercial bleach followed by 2 changes of sterile water

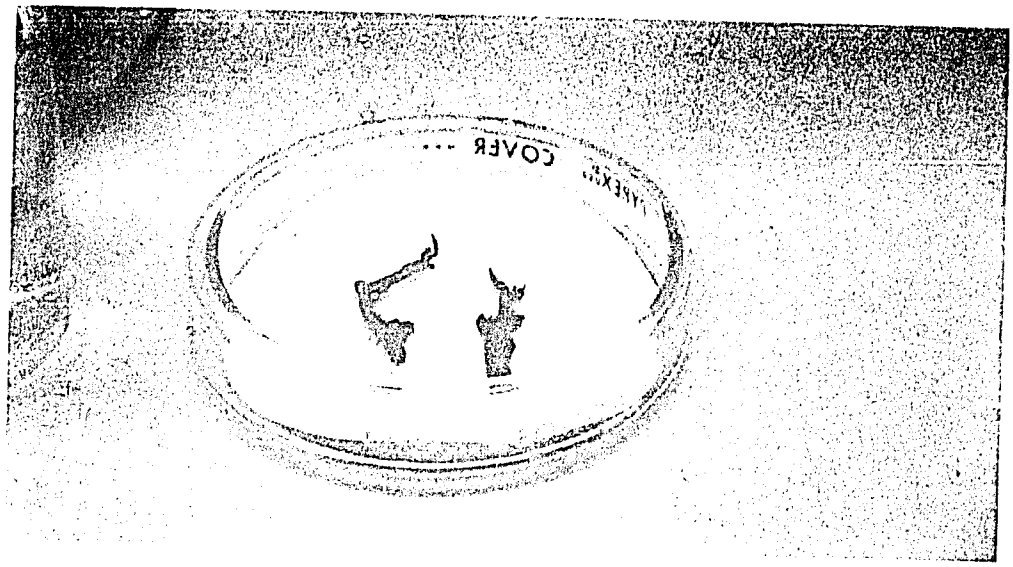

Figure 8. Explant preparation and disinfection procedures - Step 5: place the disinfected stems in a sterile Petri dish lined with filter paper to absorb excess water.

The excision procedure used in the authors' laboratory is as follows:

1. Place the dissection instruments in a 250 ml beaker filled with 75% ethanol. Line the beaker with several layers of sterile towelling or gauze cloth to protect the tips of the instruments. The mimimum needed for excision of the meristem tips are two scalpel handles with disposable blades, and one pair of fine tipped forceps. Sterilize the tools between each use by flaming in an alcohol lamp.

2. Place a disinfected stem in a sterile Petri dish under a dissecting microscope. Using the forceps, hold the stem steady while using one of the two scalpels to remove the largest of the young leaves (Figs. 9 and 10). This can be done easily by bending the leaves away from the stem with the back of the scalpel blade.

3. Remove the underlying leaf primordia by inserting the tip of the scalpel into the base of each primordium and flicking the tip of the scalpel away from the stem axis.

4. At this point, the apical dome should be visible, flanked by two or three of the youngest leaf primordia (Fig. 11). Removal of these primordia is difficult without damaging the fragile apical dome, and extreme care must be used. Their removal can be accomplished by scraping them off with the cutting edge or back edge of the scalpel blade. It is important that all leaf primordia are removed and only the apical dome (0.1 mm in depth; Fig. 12) excised in order to increase the probability of obtaining plants free of viruses.

5. The second scalpel should be reserved for removal of the apical dome after the dissection process has been completed. This ensures a sharp blade that is free from viruses and micro-organisms that may have survived the disinfection process. Hold the stem so that the apical dome can be seen in profile and using the second scalpel make a slicing cut at the base of the dome. The cut should penetrate about halfway through the tissue beneath the dome and then by using a lifting motion the dome should separate from the supporting tissue and adhere to the blade. Sometimes a second cut is needed beneath the opposite side of the dome to complete removal. The excised dome is then quickly transferred to the waiting culture tube. The dome will just be visible to the naked eye, and care must be taken to ensure that it is placed on the surface of the medium (Excision and Induction Medium; Table 3) rather than adhering to the tip of the scalpel.

It should be noted that once the dissection procedure progresses to the point of exposing the apical dome, the meristem begins to lose water rapidly. Once the dome becomes dehydrated it is difficult to remove and seldom survives. Consequently, the final steps in the excision process must be completed rapidly.

The importance of maintaining sterility of the dissection instruments cannot be over-emphasized. Keep them in 75% ethanol when not being used and always flame them before use. Keep one scalpel strictly for use in the final removal of the apical dome. It is absolutely essential to use a clean sharp scalpel for this stage. If difficulties are experienced in removing the apical dome without excessive damage, it may be advisable to try the alternative instruments (lens knife, micro-scalpel) mentioned earlier (see: Culture Preparation Area).

Incubation of Cultures

Following excision and inoculation, the cultures should be placed on a bench under a controlled lighting and temperature regime. Incubation conditions are discussed in detail in an earlier section (see: Incubation Area).

Figure 9. Shoot-tip just prior to excision. The next step in the excision process is removal of the young leaves by bending each one away from the stem.

Figure 10. Shoot-tip just prior to excision. The next step in the excision process is removal of the largest leaf primordia.

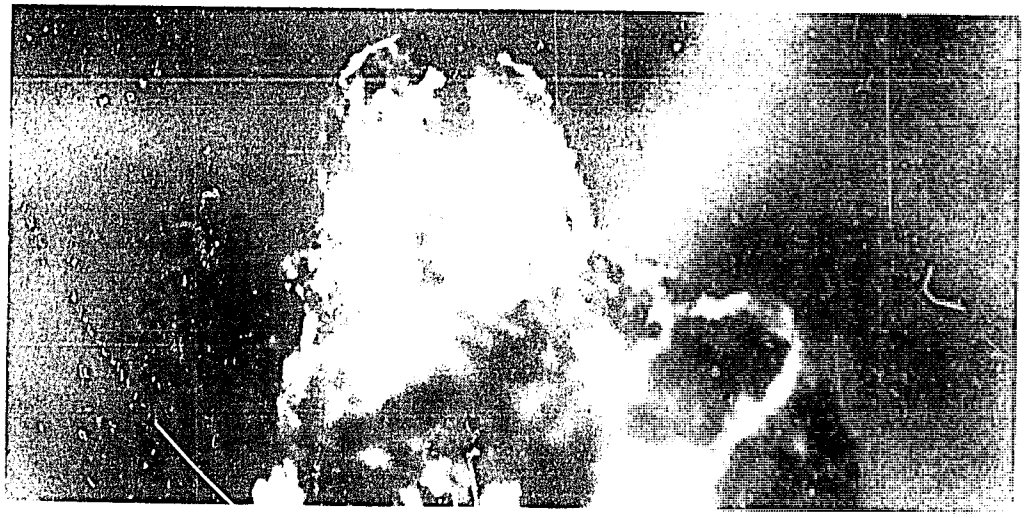

Figure 11. Shoot-tip following removal of the largest leaf primordia youngest pair of leaf primordia remain. The next step in the excision process is removal of the remaining leaf primordia in order to expose the apical dome.

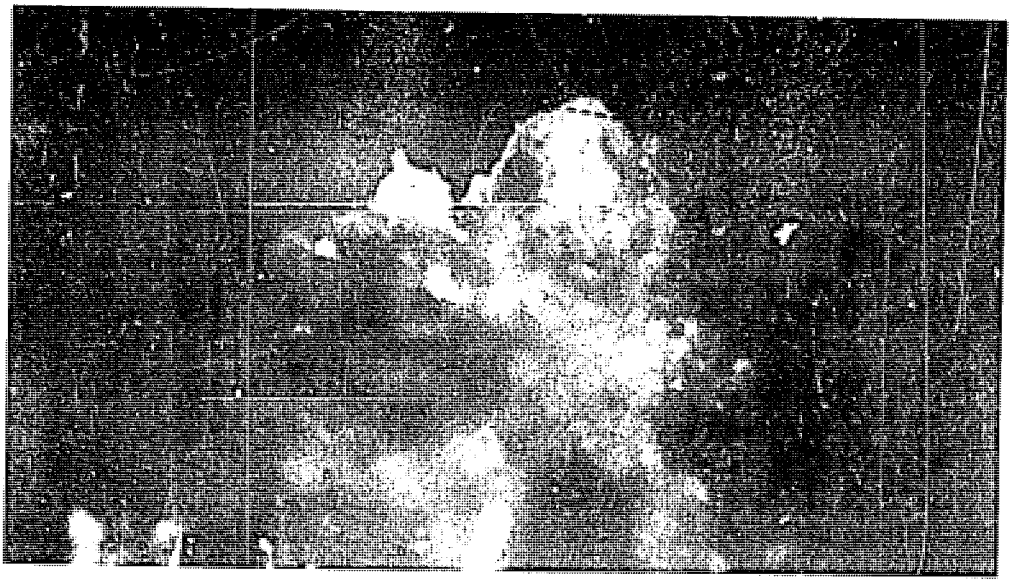

Figure 12. Shoot-tip showing the exposed apical dome. The dome is now removed and placed on to culture medium. The dotted line through the base of the apical dome indicates the proper incision point to obtain a piece of tissue approximately 0.1 mm in length.

Culture Maintenance and Plantlet Production

One week after excision, the meristem-tip cultures should be inspected under the dissecting microscope and contaminated and dead cultures discarded. If contamination exceeds 5-10%, the disinfection and excision procedures should be reviewed. Normally, 25-40% of the cultures can be expected to die as a result of dissection damage and/or too small a size of explant. Dead cultures can be recognized by their black appearance and lack of new growth.

The usual sequence of events preceding regeneration is (1) swelling of the meristem-tip, (2) callus production, (3) shoot development and, finally (4) root development, (Fig. 13). This sequence can take from 1-4 months. However, nutrient deficiency, medium dehydration and accumulation of secondary metabolites may cause the growth of meristems to slow down after 4-6 weeks. Consequently, it is necessary to transfer the meristems to fresh medium at intervals of no more than 4 weeks.

The meristem-tips are maintained on the Excision and Induction Medium until the first signs of shoot development appear. They are then transferred immediately to Regeneration Medium (Table 3).

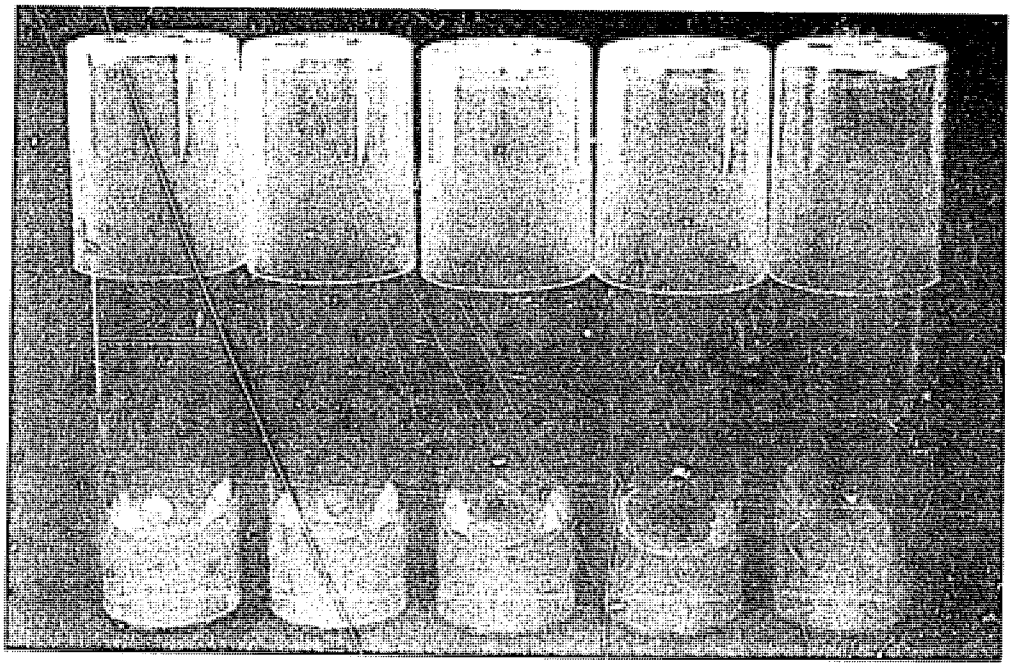

Figure 13. Developmental sequence of meristem-tips beginning at excision and ending with a plantlet. From left to right: 1 week, slight swelling; 3 weeks, callus development; 5 weeks, leaf primordia appear; 6 weeks, shoot growth; 8 weeks, root growth. The development time indicated here is an average and will vary with incubation conditions and genotype.

Growth regulators, as in Induction and Regeneration Medium, must apparently be present to induce shoot production but, following the induction step, the presence of exogenous growth regulators (especially BAP) slows down further growth. Cultures transferred from Excision and Induction Medium to Regeneration Medium whilst still at the callus stage will often produce shoots within a few days. However, because it is impossible to observe the induction process without sacrificing the culture, transfer to Regeneration Medium must await the appearance of a visible shoot. Normal root development follows the development of a normal shoot regardless of whether growth regulators are present or not, but Regeneration Medium is very suitable for root production.

The average time needed to regenerate plantlets from meristems varies widely with genotype, explant size and culture conditions. When the original explant is a 0.1 mm apical dome, plants should not be expected before 5 weeks have elapsed. In some genotypes it may take 3-4 months before the first plantlets regenerate.

In Vitro Propagation of Plantlets

It is important to maintain the plantlets regenerated from meristem-tips in culture while virus indexing is being carried out in order to prevent re-infection. This can be accomplished by taking several single node cuttings from a regenerated plantlet. The cuttings are placed on Regeneration Medium and allowed to develop into plantlets under the same environmental conditions as used for the original cultures. Some of the plantlets can then be taken out of culture, established in soil and used for virus indexing. The remaining plantlets are maintained in culture by repeating the nodal propagation system as often as necessary. Following virus indexing, the plantlets remaining in culture can serve as a source of stock plant material for provision of material for shipment to other laboratories or germplasm repositories.

The same procedure may be used to multiply valuable clones and provide stocks for *in vitro* storage of germplasm (see: *In Vitro* Conservation).

In Vivo Establishment of Plantlets

Regenerated plantlets are ready to be established in the soil when they have two or three leaves and at least one strong root. As indicated earlier, a 1:1 sand:soil (volume:volume) mixture is the best planting medium. Avoid soil mixtures with high peat content, since they often lead to slower growth and a higher death rate (Alconero *et al.*, 1975). The soil mixture should be sterilized (but see below). The following procedure utilizes the improvised planting containers described earlier (see: Plant Establishment Area) and shown in Fig. 2.

Place a piece of filter paper or paper towel in the bottom of the pot cup to prevent the sand mixture from leaking out. Fill the cup with the sterile soil mixture and wet thoroughly. Remove the plantlet from the culture tube and transplant into the cup, making sure that the roots extend well into the soil. It may be beneficial to rinse away adhering agar. As an alternative to using sterilized soil, the roots may be dipped in a fungicide and then planted into non-sterile soil.

After transplanting, water the plantlet well to ensure that the soil mixture makes good contact with the roots. Be careful not to allow the soil to cover the growing point. Finally, place the clear covering cup over each plantlet to prevent dehydration. Place the plantlets in a shaded glasshouse or growth chamber. Do not expose them to direct sunlight until the hardening process, described in the next paragraph, has been completed.

Figure 14. System for establishing regenerated plantlets in soil. Plantlets are removed from the tubes and placed in a covered pot (left). After 1 week the cover is propped up to begin the hardening process (centre). After an additional week, the cover is removed completely (right). The plants are ready to be placed in a shaded glasshouse.

Approximately 1 week after transplanting, prop the plastic covering cup part way open to reduce the internal humidity and allow further acclimatization. Two weeks after transplanting, the covers can be removed entirely and the plantlets exposed to full sunlight (Fig. 14). Ensure that the soil always remains moist. It may also be beneficial to apply a dilute solution of water-soluble fertilizer at the time the cover is removed. Within a month of removing the plantlets from culture, they should be large enough to transplant into larger pots or into the field.

In Vitro Conservation

Two approaches are available for the storage of *in vitro* cultures: slow growth, appropriate for medium-term storage in the *in vitro* active genebank (IVAG); and cryopreservation in liquid nitrogen for long-term storage in the *in vitro* base genebank (IVBG; see: IBPGR, 1986).

Slow Growth

Shoot-cultures of sweet potato appear to be amenable to growth-limiting treatments. According to published reports (IITA, 1981; Jarret *et al.*, 1986; Ng and Hahn, 1985; Wheelans and Withers, 1984; see: APPENDIX I) and the unpublished work of the authors, a consistently successful treatment is to culture in the presence of mannitol at approximately 3% and reduce the growth temperature to 16°C or 18°C. A higher level of mannitol may be effective in reducing growth at the normal incubation temperature, thus allowing stored cultures to be maintained alongside those in normal growth. Culture at 18°C in the presence of the growth retardant 'Alar' (40 mg/l) and under low light (reduced from 3 klux to 1 klux) has also been found to be satisfactory (personal communication: S. Salazar and V.M. Villalobos). Other modifications to the culture medium (reduction in the concentration of salts or sucrose; addition of abscisic acid) may be worth testing.

Storage in slow growth for up to 2 years appears to be feasible (e.g. Ng and Hahn, 1985) but cultures should be checked for contamination or necrosis at frequent intervals, discarding or transferring as necessary. Experience with other species indicates that a brief period at the normal culture temperature is advisable to re-establish growth after transfer.

Cryopreservation

Latta (1971) reported survival of suspension cultured cells of a species of *Ipomoea* after exposure to -40°C which indicates some low temperature tolerance under appropriate protective conditions. However, the method described would not be suitable for long-term storage. Attempts by the authors and others to cryopreserve meristems or shoot-tips of sweet potato have failed but studies are continuing.

Potential Problems - Troubleshooting

Three common problems encountered in the culture procedures are contamination of cultures, high mortality of excised meristem-tips, and lack of regeneration.

Contamination

If a large number of cultures are contaminated following excision, the first step is to determine the source of contamination. It can often be detected by looking for patterns of contamination among the cultures. When contamination appears in a series of tubes inoculated consecutively, it is probably due to failure to sterilize the dissecting instruments properly between explants. If the contamination appears only in tubes containing certain genotypes or meristem-tips of plant material from similar locations, the source is likely to be the plant material itself.

If the source of contamination is determined to be the dissecting instruments, the problem can usually be resolved by making sure that they are properly flamed between dissections. It is also advisable to make sure that all working surfaces are completely sterile.

If the source of contamination can be traced back to the plant material itself, several steps can be taken to reduce the problem. Firstly, ensure that an effective insect control programme is followed for plant material kept in a glasshouse. Also, plant the parent material in sterilized sand. If excessive contamination persists, increase the time the stems are soaked in sodium hypochlorite to 25 minutes and follow this with a 5 second dip in 75% ethanol. This treatment will give more complete disinfection but will also increase the death rate of the excised meristem-tips.

Death of Excised Meristem-tips

A 25-40% death rate of the newly excised meristems can be considered normal. However, if the death rate reaches higher proportions, much effort and plant material is wasted.

Meristem death is most commonly caused by excessive damage to the apical dome during the excision process, which should be done very carefully. If the dome is damaged excessively by contact with the dissecting instruments, chances of survival are reduced. A second reason for damage is dehydration. Once the meristem is exposed, it must be removed quickly and placed on to the culture medium.

The sucrose concentrations in the culture medium can have a critical effect on meristem-tip survival. Concentrations below 45 g/l will lead to a higher percentage of dead meristems.

Floating meristem-tips on liquid media until they have been observed to swell may be beneficial. Surface tension will support the explant (R.L. Jarret, unpublished observation).

Lack of Regeneration

The ability of sweet potatoes to regenerate under the culture conditions specified here varies widely between genotypes. Some of the common problems experienced in the authors' laboratory, and the steps taken to overcome them, are as follows:

Symptom: No swelling or production of callus by the meristem-tips.

Possible solutions: If the lack of growth is exhibited by all of the genotypes in culture the problem probably lies with the medium. If the medium formulation has been successful in the past, prepare new stock solutions, especially of the growth regulators. Stock solutions in which there are precipitates should be discarded. If a lack of growth is exhibited by only one or a few genotypes increase the concentration of NAA in the medium to 0.05 mg/l. A number of other media formulations have been published (Table 5), that may be used for material that fails to respond to the medium recommended here.

Symptom: Callus growth but no shoot production.

Possible solutions: Make sure the meristem-tips were taken from healthy plants. Those taken from even the most easily regenerated genotypes may not produce shoots if the parent plants are not healthy.

Vary the concentration of BAP. The optimum concentration of BAP has been found to be genotype dependent, more so than any other single medium component, and was found to vary from 0.10-0.50 mg/l. Although the 0.30 mg/l concentration of BAP in the Excision and Induction Medium (Table 3) is appropriate for most genotypes, some may have to be exposed to a higher or lower concentration (possibly as low as 0.05 or as high as 1.00 mg/l). Again, alternative medium formulations may be tried (Table 5).

If no regeneration occurs after following the previously described steps, as a last resort use a slightly larger meristem-tip. A larger explant, e.g. the apical dome along with the youngest two or three leaf primordia, will be less demanding in its culture conditions; successful regeneration will probably occur without special treatment. However, it must be realized that by removing a larger portion of the shoot-tip, the probability of regenerated plants' being virus-infected will be increased. This will be revealed by indexing for known viruses (see: VIRUS INDEXING PROCEDURES).

Table 5. Alternative media formulations for sweet potato meristem-tip culture 1/, 2/

Factor 3/	Love and Rhodes 4/	Elliott (1969)	Alconero et al. (1975)	Frison and Ng (1981)	Salazar (1983)	Kuo et al. (1985)
M&S Salts	+	+	+	+	+	+
Sucrose	50,000	30,000	30,000	30,000	30,000	30,000
Myo-inositol	100	100 5/	100	200	200	100
Nicotinic acid		0.5 5/		1	0.5	0.5
Pyridoxine.HCl		0.5 5/		1	0.5	0.5
Glycine		2 5/		4	2	2
Thiamine.HCl	2	2 5/	2	0.2	0.1	0.1
Adenine sulphate				80		
Na_2EDTA (additional)	37.5					
Gibberellic acid (GA_3)		10 6/			0.1	
NAA (Exc. and Ind.) 7/	0.05	1 6/				1
(Regeneration)	0					
Indoleacetic acid (IAA)			8/	0.2	0, 0.1, 0.5	
BAP (Exc. and Ind.)	0.3		8/	0.5		
(Regeneration)	0					
Kinetin		0.1 6/			0, 0.1, 0.5	1
pH	5.2	9/	5.65	5.7	5.7	5.7

1/ See original publications for further details.
2/ All formulations may be prepared with or without agar (7 g/l).
3/ All factors are expressed in mg/l.
4/ Formulations used in this manual.
5/ Thiamine.HCl may be used instead of myo-inositol, nicotinic acid, pyridoxine.HCl and glycine.
6/ Media continuing different combinations of growth regulators were compared.
7/ See: Culture Maintenance and Plantlet Production for explanation of use of 'Excision and Induction' and 'Regeneration' media.
8/ Variable.
9/ No pH value quoted.

Transportation of Cultures

Plantlets regenerated from meristem-tips can easily be transported locally and internationally (Roca, 1980: Roca et al., 1979). It is emphasized that only those indexed and shown to be free of all known pathogens should be distributed. The following steps and precautions should be taken in order to make delivery as trouble free as possible.

1. Make sure that the cultures to be shipped are free from bacterial, fungal or viral infections. Virus indexing should be completed and the method used and results obtained stated on the package.

2. If the cultures are to be shipped internationally, have the plantlets inspected by the appropriate quarantine officials.

3. Prepare the cultures for distribution. Choose plantlets with a well-developed root system and a large shoot. These will survive rough handling better than will less well-developed cultures. Make sure of the cap of the culture vessel is securely fastened with 'Parafilm' or tape.

4. If the cultures are to be hand carried only minimal packing is necessary and they can be delivered in the tubes in which they were grown. Wrap the tubes separately in paper towels or cheese cloth to prevent breakage and use any suitable container for transport. Keep the tubes upright, if possible. Hand delivery is the method of choice.

5. If the plantlets are to be transported by mail or by air, special packing precautions must be followed. Several weeks in advance, transfer the plantlets to a medium solidified with 1% agar in the smallest culture tubes feasible. Allow the plantlets to establish a strong root system in the agar. The medium is less likely to break up in transit if culture tubes with a restriction near the top of the medium are used. Immediately before distributing, slide a sterile, tightly-fitting cotton plug into each tube until it just contacts the shoots. This will prevent the shoot from breaking away from the roots. Pack the tubes tightly into a sturdy container. Polystyrene or other suitable insulated containers should be used to buffer against temperature extremes. Label the package as live material to prevent adverse handling and consign by the fastest method possible, preferably by air.

6. Include with each shipment a complete list of the plant material, a phytosanitary certificate, if applicable, and a set of instructions explaining the procedure for further *in vitro* propagation or *in vivo* establishment of plantlets at the receiving station.

If the necessary precautions are taken, sweet potato plants derived from meristem-tips can successfully be distributed world-wide. As long as the plantlets are not subjected to temperature extremes (below 10°C or above 40°C) and/or extended periods of darkness in transit, establishment at the receiving station will be quick and successful.

The system described above is obviously not the only one that can be used, nor will it work in every situation. It is designed primarily for the transport of regenerated meristem-tips or *in vitro* propagated plantlets. However, if the same basic precautions are followed and small trial shipments used to identify workable systems, cultures can be distributed at almost any stage of growth.

VIRUS INDEXING PROCEDURES

Introduction

Virus indexing of sweet potato is accomplished by grafting shoots from plants to be tested on to indicator plants (Frison and Ng, 1981). Ipomoea nil 'Scarlet O'Hara' and/or I. setosa, two wild relatives of sweet potato, are most commonly used. If virus is present, spotting, vein clearing, chlorotic mottle, epinasty, or stunting will occur on the indicator plant. A complementary grafting technique using susceptible pre-infected sweet potato seedling clones has proved useful in detecting sweet potato viruses in Nigeria (Rossel and Thottappilly, 1985).

Virus indexing procedures for sweet potatoes are changing rapidly as new tests, including molecular techniques such as nucleic acid hybridization, are developed. Consequently, the indexing procedure outlined here may be outdated within a few years. However, it is currently in wide use and is the best practical method available for detection of most known viruses in sweet potatoes. Researchers who use this manual should be aware of the continuing need to review current literature in search of improved techniques. Further information on current procedures may also be obtained from Dr. J.W. Moyer, Associate Professor, Department of Plant Pathology, North Carolina State University, Raleigh, NC 2769-7616, USA; Dr. J. Foster, USDA-APHIS-PPQ, US Plant Introduction Station, Glenn Dale, MD 20769, USA or from virologists at AVRDC, P.O. Box 42, Shanhua, Taiwan 741, China; CIP, Apartado 5969, Lima, Peru or IITA, P.O. Box 5320, Ibadan, Nigeria.

Meristem-tip culture cannot guarantee freedom from virus infection and the inability to detect virus symptoms in indicator plants does not mean that the indexed sweet potato plants are completely free from all viruses. The procedure has been used to detect several major viruses (Alconero et al., 1975; Frison and Ng, 1981), but it may not be effective for detecting others. Also, it does not have a very high degree of sensitivity.

Materials and Equipment

The virus indexing system described here is relatively simple, with only a minimal amount of equipment and materials needed. Plant material and grafting tools are the basic requirements.

Plant Material

The sweet potato plants to be indexed for virus should appear healthy and be fairly large. Five nodes, each with a fully expunded leaf, are used from each plant tested.

The indicator plants, I. nil 'Scarlet O'Hara' and I. setosa can be grown from seed. Seed of I. setosa must be maintained by the user, but can be obtained initially from Dr. A. Jones (USDA Vegetable Breeding Laboratory, Charleston, South Carolina), Dr. J.W. Moyer (address above) or from many other sweet potato breeders. Seed of I. nil 'Scarlet O'Hara', an ornamental, can be obtained from many seed companies.

Both sweet potato and indicator plants can be maintained in a glasshouse. Insect control equipment is needed because it is important to prevent the spread of viruses from infected to non-infected plants. A spraying programme should be developed and strictly followed.

Grafting Tools

Essential tools for grafting the sweet potato stems on to the indicator plants include a scalpel with disposable blades, an alcohol lamp for sterilization purposes, and 'Parafilm' to seal the graft. Cheesecloth is useful for protecting and shading the newly grafted stems.

Methodology

Preparation of Plant Material

Seed of the indicator plants should be planted 3-4 weeks before the grafting is to be done. Seeds must first be scarifled by soaking in concentrated sulphuric acid for 20-25 minutes, and then rinsed three times with tap water. Plant seeds in a 1:1 sand:soil (volume:volume) mixture in individual pots. The indicator plants are ready for grafting when they have two fully expanded leaves. Allowing the indicator plants to become too old may reduce the visibillty of virus symptoms. If a large number of sweet potato plants are to be indexed over an extended period of time, it is recommended that seed of indicator plants be sown at least at weekly intervals to ensure a constant supply at the proper stage of growth.

Sweet potato plants that are to be indexed can be produced by in vitro nodal propagation of the original meristem cultured plantlet as explained above (see: In Vitro Propagation of Plantlets). As advised in that section, additional plantlets should also be maintained in culture to be used as stock plants after the indexing procedure is complete. Each sweet potato plant that is used for the indexing procedures should be established in soil and allowed to develop until it has a minimum of five nodes with fully expanded leaves.

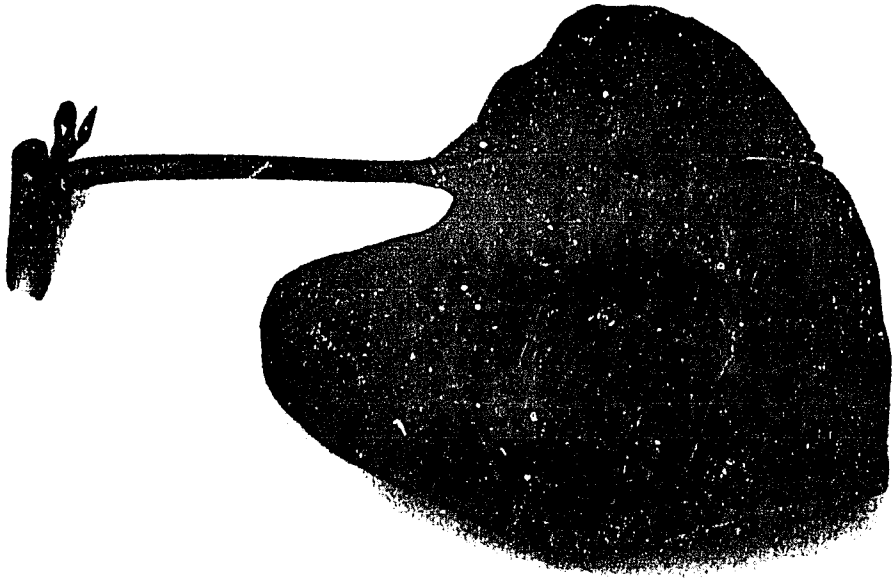

Figure 15. Sweet potato node with attached leaf, ready to be grafted on to an indicator plant for virus indexing.

Grafting Procedure

Begin the grafting procedure by cutting five, 3-4 week old indicator plants back to two true leaves. Then, remove a branch from the sweet potato plant to be indexed. Cut the branch into five sections, each containing a node with a fully expanded leaf attached. Fig. 15 shows a sweet potato node section that is ready for grafting. Wedge graft each node section on to a separate indicator plant as shown in Fig. 16. Wrap the graft with 'Parafilm' to prevent desiccation (Fig. 17). Protect the scion from direct sunlight by laying a piece of cheesecloth over the graft and the adjoining leaves for 2-3 days.

Figure 16. Sweet potato node being wedge grafted on to the stem of an I. setosa indicator plant, just below the first leaf.

By grafting five nodes from each sweet potato plant on to five separate indicator plants, the chances of detecting symptoms of virus infection are increased. Symptoms on the indicator plants are sometimes sporadic and often short-lived. Some control grafts should be made to provide comparisons: graft some stems from one indicator plant on to another indicator plant, and also graft a sweet potato plant known to be virus-infected on to some indicator plants.

Be sure to avoid cross-contamination of plants during the grafting process, by dipping the scalpel used for cutting plant material in 75% ethanol and flaming between every graft.

Figure 17. Completed graft of a sweet potato node on to an indicator plant.

Indexing Procedure

The severity of the virus symptoms and the length of time before they appear on the indicator plants are dependent upon the type and amount of virus present in the scion. As indicated earlier, the most common symptoms of virus infection are spotting, vein clearing (chlorosis), chlorotic mottle, epinasty and stunting. The symptoms are most severe on the leaves of the indicator plant nearest to the graft. Symptoms may appear on I. nil within a week. Symptoms on I. setosa (Fig. 18) usually appear within 2-4 weeks. It is important to monitor the indicator plants at frequent intervals because they may only show symptoms for only a short period of time.

Indexing by using I. setosa and I. nil as indicator plants has both advantages and disadvantages for virus detection. The technique is non-specific and detects nearly all of the viruses that infect sweet potatoes. However, it is slow, requiring up to several

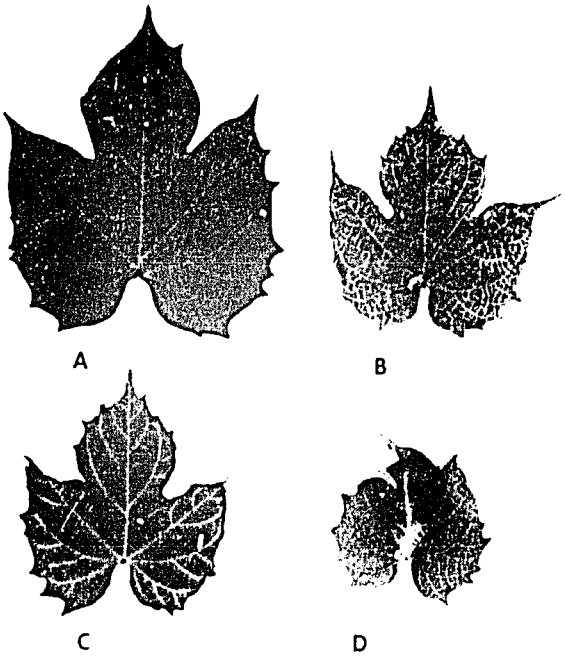

Figure 18. Symptom progression in Ipomoea setosa infected with sweet potato feathery mottle virus: (A) Healthy leaf; (B) Initial symptom consisting of veinal chlorosis; (C) Subsequent leaves with chlorotic vein-banding restricted to major veins; (D) chlorotic vein-banding adjacent to midrib; epinasty. New leaves are frequently symptomless (from Moyer and Kennedy, 1978).

months to complete, and lacks sensitivity. The lack of sensitivity is the reason why five grafts are recommended for every sweet potato plant indexed. It is necessary to repeat the indexing of negatively indexed sweet potato plants two more times before declaring them free from known viruses (Frison and Ng, 1981).

Once a sweet potato plant is determined to be free from known viruses, it is a simple procedure to propagate it clonally through in vitro culture (see: In Vitro Propagation of Plantlets).

Plantlets so produced and distributed to germplasm repositories or to research laboratories should be accompanied by a label declaring them to be free from known viruses. The method used for virus indexing should also be included.

Further details of practical procedures and precautions to be used in the distribution of cultures are given in an earlier section (see: Transportation of Cultures).

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APPENDIX

A summary of entries on sweet potato and other Ipomoea species from the IBPGR In Vitro Conservation Databases (Wheelans and Withers, 1984) is given below. Each entry includes four fields of data: NAME and ADDR, containing the name and address of the contact; DATE, indicating the year of the survey to which information was submitted (1980/81, 1983 or 1985; there are some entries covering more than one year), and APPL, listing the interests of the contact and intended applications of the in vitro work. Full details of database entries, covering propagation, characterization, disease indexing, germplasm storage and exchange, and problems experienced, can be requested via IBPGR Headquarters.

NAME ANDERSON, J.O.
ADDR Department of Plant Science, University of Arizona, Tuscon, Arizona 85721, USA.
DATE 1980/81
APPL Fundamental research, rapid clonal propagation, germplasm storage.

NAME ANON
ADDR Chia-Yi Agricultural Experimental Station, 2 Min-Chun Road, Chia-Yi, 600 Taiwan, Republic of China.
DATE 1983
APPL Rapid clonal propagation, virus or other pathogen elimination, germplasm storage, germplasm exchange.

NAME BATISTA TEIXEIRA, J.
ADDR CENARGEN-EMBRAPA, AVW5N, Parque Rural, PO Box 10.2372, Brasilia, Brazil.
DATE 1983
APPL Fundamental research, virus or other pathogen elimination, germplasm storage.

NAME BONNEL, E.
ADDR CIRAD - IRAT, 97487 Saint-Denis Cedex, Reunion Island, (France).
DATE 1985
APPL Rapid clonal propagation, virus or other pathogen elimination, germplasm storage, germplasm exchange.

NAME CLARK, C.A.
ADDR Department of Plant Pathology & Crop Physiology, LSU, Baton Rouge, LA 70803-1720, USA.
DATE 1985
APPL Rapid clonal propagation, virus or other pathogen elimination, genetic manipulation

NAME DELGADO, G.E.
ADDR Universidad Nacional Pedro Ruiz Gallo, Apartado 48, Lambayeque, Peru.
DATE 1985
APPL Rapid clonal propagation, germplasm storage, germplasm exchange.

NAME DIRECTOR
ADDR Zambia, Department of Agriculture, Research Branch, Mount Makulu Research Station, Box 7, Chilanga, Zambia.
DATE 1980/81
APPL Rapid clonal propagation, virus or other pathogen elimination, germplasm storage.
DATE 1983
APPL Rapid clonal propagation, virus or other pathogen elimination, germplasm storage.

NAME DODDS, J.
ADDR CIP, Apartado Postal 5969, Lima, Peru.
DATE 1985
APPL Rapid clonal propagation, virus or other pathogen elimination, germplasm storage, germplasm exchange, genetic manipulation.

NAME FERREIRA, D.I.
ADDR Horticultural Research Institute, Department of Agriculture and Fisheries, Private Bag 293, Pretoria, Republic of South Africa.
DATE 1980/81
APPL Virus or other pathogen elimination.

NAME FRISON, E.A.
ADDR International Institute of Tropical Agriculture, Oyo Road, Ibadan, Nigeria (present address: IBPGR Headquarters, FAO, via delle Terme di Caracalla, 00100 Rome, Italy).
DATE 1980/81
APPL Rapid clonal propagation, virus or other pathogen elimination, germplasm storage, germplasm exchange.

NAME GAMA, MARIA I.C.S.
ADDR CENARGEN/EMBRAPA, SAIN-PARQUE RURAL, C.P. 102372, 70770 Brasilia DF, Brazil.
DATE 1985
APPL Virus or other pathogen elimination.

NAME GU, SHU-RONG
ADDR Institute of Botany, Academia Sinica, Beijing, China.
DATE 1980/81
APPL Rapid clonal propagation, virus or other pathogen elimination, germplasm storage.

NAME HENSHAW, G.G.
ADDR Department of Plant Biology, University of Birmingham, PO Box 363, Birmingham B15 2TT, UK. (Present address: Department of Biology, University of Bath, Claverton Down, Bath, Avon, UK).
DATE 1980/81
APPL Rapid clonal propagation, germplasm storage.

NAME JACKSON, G.V.H.
ADDR Dodo Creek Research Station, Ministry of Home Affairs & National Development, PO Box G.13, Honiara, Solomon Islands.
DATE 1983
APPL Germplasm exchange.
ADDR Plant Protection Project, U.N.D.P., Private Mail Bag, Suva, Fiji (present address: 17 Hyde Road, Richmond, Surrey, UK).
DATE 1985
APPL Virus or other pathogen elimination, germplasm exchange.

NAME JARRET, R.L.
ADDR Apartado 78, CATIE, Turrialba, Costa Rica (present address: USDA-ARS, Regional Plant Introduction Station, Experiment, Georgia, USA).
DATE 1983
APPL Fundamental research.

NAME KAMAU, H.N.
ADDR Plant Quarantine Station, Kenya Agricultural Research Institute, Headquarters Muguga, PO Box 57811, Nairobi, Kenya.
DATE 1983
APPL Rapid clonal propagation.
DATE 1985
APPL Rapid clonal propagation, germplasm exchange.

NAME KARTHA, K.K.
ADDR Prairie Regional Laboratory, (Plant Biotechnology Institute), National Research Council, Saskatoon, Sask., Canada S7N OW9.
DATE 1980/81
APPL Rapid clonal propagation, virus or other pathogen elimination, germplasm storage.

NAME KAVI KISHOR, P.B.
ADDR Department of Botany, Kakatiya University, Warangal-506 009, A.P.,
India.
DATE 1985
APPL Fundamental research, rapid clonal propagation, virus or other pathogen elimination, secondary product synthesis.

NAME LAM, T-H.
ADDR Department of Botany, University of Hong Kong, Hong Kong.
DATE 1980/81
APPL Secondary product synthesis.

NAME LI, LANG
ADDR The Director, Chia-Yi Agricultural Experiment Station, 2 Min-Cheng Road, Chia-Yi 600, Taiwan, China.
DATE 1985
APPL Virus or other pathogen elimination, germplasm storage and germplasm exchange.

NAME LINTON, P.
ADDR Dodo Creek Research Station, P.O. Box G.13, Honiara, Solomon Islands.
DATE 1985
APPL Virus or other pathogen elimination, germplasm storage and germplasm exchange.

NAME LITZ, R.E.
ADDR University of Florida, Agricultural and Research Centre, 18905 SW 28th Street, Homestead, Florida 33031, USA.
DATE 1980/81
APPL Rapid clonal propagation, germplasm, storage, germplasm exchange.

NAME LOPEZ-GUTIERREZ, F.
ADDR Centro de Investigaciones Biologicas de Baja California Sur, P.O. Box 128, 23000 La Paz, Baja California Sur, Mexico.
DATE 1985
APPL Selection for salt tolerance.

NAME MARTIN, F.W.
ADDR Federal Experimental Station, USDA, Mayaguez, Puerto Rico, USA (now retired).
DATE 1980/81
APPL Rapid clonal propagation, virus or other pathogen elimination, germplasm storage, germplasm exchange.

NAME MROGINSKI, L.
ADDR Instituto de Botanica del Nordeste, Casilla de Correo 209, 3400 Corrientes, Argentina.
DATE 1983
APPL Virus or other pathogen elimination, germplasm storage.

NAME MULLER, L.E.
ADDR CATIE, 7170 Turrialba, Costa Rica.
DATE 1985
APPL Fundamental research.

NAME NEL, DOROTHEA D.
ADDR Horticultural Research Institute, Department of Agriculture and Fisheries, Private Bag 293, Pretoria, Republic of South Africa.
DATE 1980/81
APPL Virus or other pathogen elimination.

NAME NG, S.Y.
ADDR International Institute of Tropical Agriculture, Oyo Road, PMB 5320, Ibadan, Nigeria.
DATE 1983
APPL Rapid clonal propagation, virus or other pathogen elimination, germplasm storage, germplasm exchange.

NAME PALMER, C.E.
ADDR Department of Plant Science, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2NZ.
DATE 1983
APPL Fundamental research.

NAME QUAK, F.
ADDR Research Institute for Plant Protection, PMB 9060, 6700 WG Wageningen, The Netherlands.
DATE 1985
APPL Virus or other pathogen elimination, germplasm storage and germplasm exchange.

NAME REY, H.
ADDR Instituto de Botanica del Nordeste, Casilia de Correo 209, 3400 Corrientes, Argentina.
DATE 1983
APPL Virus or other pathogen elimination, germplasm storage.

NAME SALAZAR, S.S.
ADDR Unidad de Recursos Fitogeneticos (Genetic Resources Unit), Apartado 15, CATIE, 7170 Turrialba, Costa Rica.
DATE 1983
APPL Rapid clonal propagation, virus or other pathogen elimination, germplasm storage, germplasm exchange.

NAME SALGADO-GARCIGLIA, R.
ADDR Centro de Investigaciones Biologicas de Baja California Sur, P.O. Box 128, 23000 La Paz, Baja California Sur, Mexico.
DATE 1985
APPL Selection for salt tolerance.

NAME SASAKI, T.
ADDR National Food Research Institute, Tsukuba Science City, Ibaraki 305, Japan.
DATE 1983
APPL Fundamental research.

NAME SKIRVIN, R.M.
ADDR University of Illinois at Urbana-Champaign, Agricultural Experiment Station, Mumford Hall, Urbana, Illinois 61801, USA.
DATE 1985
APPL Genetic manipulation

NAME SONDAHL, M.R.
ADDR Departmento de Genetica, Instituto Agronomico, Caixa Postal 28, 13100 Campinas, SP, Brazil. (Present address: DNA Plant Technology Corporation, 2611 Branch Pike, Cinnaminson, NJ 08077, USA).
DATE 1980/81
APPL Rapid clonal propagation, virus or other pathogen elimination.

NAME THI THANH TUYEN, N.
ADDR Department of Horticulture, VISCA, Leyte 7127-A, The Philippines.
DATE 1985
APPL Rapid clonal propagation, virus or other pathogen elimination, germplasm exchange.

NAME TSAY, H-S.
ADDR Taiwan Agricultural Research Institute, 189 Chung-cheng Road, Wan-feng, Wu-feng, Taichung, China.
DATE 1985
APPL Virus or other pathogen elimination, germplasm storage and haploid production.

NAME VAN RENSBURG, J.G.J.
ADDR Horticultural Research Institute, Private Bag X293, Pretoria 001, Republic of South Africa.
DATE 1983
APPL Virus or other pathogen elimination.
DATE 1985
APPL Virus or other pathogen elimination.

NAME WANG, P.J.
ADDR Institute of Botany, Academia Sinica, Nankang, Taiwan, China.
DATE 1980/81
APPL Germplasm storage.

NAME WANG, HAN,
ADDR Agricultural College of Beijing, China.
DATE 1980/81
APPL Rapid clonal propagation, virus or other pathogen elimination, germplasm storage.

NAME ZETTLER, F.W.
ADDR Plant Pathology Department, University of Florida, Gainesville, Florida 32611, USA.
DATE 1980/81
APPL Rapid clonal propagation, virus or other pathogen elimination.