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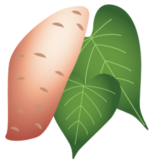
Growing healthy sweetpotato

Best practices for producing planting material



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Best practices for producing planting material

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Cover: Healthy, vigorous pathogen-tested sweetpotato vines grown in a screen house. (Photo: Sandra Dennien)

Foreword

In the mid 1980s, the Australian Centre for International Agricultural Research (ACIAR) funded a pioneering program showing how sweetpotatoes from the South Pacific region and Asia could be grown free from viruses. The program was implemented by the then Victorian Government Institute of Plant Sciences.

Although the results were spectacular, the program was not widely taken up, because it was believed that reinfection by viruses during the growing process would be too rapid for the technology to be of much practical benefit to growers. This work focused on the science of the problem and not on adaptation in the field.

More recently, however, a number of countries—Australia, China, the Philippines, South Africa and the United States—have had outstanding success with similar, but greatly simplified, pathogen-tested (PT) schemes for sweetpotato. These schemes have highlighted many times the detrimental effect of viruses on yield, and shown how growers can benefit from using healthy planting material.

Armed with this experience, ACIAR and its partners decided to look again at the technology to see how it could benefit Pacific island countries, particularly countries trying to cope with rapid population increase, declining soil fertility and climate change, all of which can adversely affect food security. ACIAR believed the technology should be field tested again with a greater emphasis placed on participation to try to drive adoption of the technology.

The methods were trialled with commercial growers in the highlands of Papua New Guinea. This area was chosen because the cooler temperatures would, it was hoped, result in a slower rate of virus reinfection. The highlands have a greater concentration of commercial growers and it was believed that

these growers would be quicker to adopt the technology and therefore help spread it wider in their local communities. The result was a simple, inexpensive and effective method of testing for sweetpotato viruses and, most importantly, for keeping vines free from reinfection. The methods are explained in this manual.

Importantly, this was the first time that all aspects of a PT scheme were conducted in a Pacific island country, and implemented by local scientists and extension personnel.

Nick Austin
Chief Executive Officer, ACIAR

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The methods presented in this manual were developed as part of ACIAR projects SMCN/2004/071 (*Reducing pest and disease impact on yield in selected Papua New Guinea sweetpotato production systems*) and PC/2010/026 (*Validating and documenting a strategy for producing virus-free sweetpotato planting material in Papua New Guinea*).

Acronyms and abbreviations

ACIAR	Australian Centre for International Agricultural Research
CIP	International Potato Center
DNA	deoxyribonucleic acid
ELISA	enzyme-linked immunosorbent assay
FTA	paper-based system to fix and store nucleic acids from fresh tissues
GPS	global positioning system
GRF	Gatton Research Facility
NCM	nitrocellulose membrane
PCR	polymerase chain reaction
PT	pathogen tested
RNA	ribonucleic acid
TBS	Tris-buffered saline
T-TBS	Tween–Tris-buffered saline
UV	ultraviolet

Units of measure

C	Celsius
cm	centimetre
g	gram
L	litre
lux	international system unit for illumination
mL	millilitre
mm	millimetre
μL	microlitre
ppm	parts per million (equivalent to μL per L)

Other terms

The underground food storage organ of the sweetpotato is often referred to as a 'tuber'. This is incorrect as a tuber is a specialised organ, while a sweetpotato is not a specialised organ but a modified root. In this manual, we use the terms 'root' and 'storage root' to refer to the sweetpotato food storage organ.

About this manual

This manual is aimed at researchers and technicians who may be new to pathogen-tested (PT) schemes for sweetpotato (*Ipomoea batatus*). It updates a previous publication (Beetham and Mason 1992) on the subject for the Pacific islands, and shows how simple the process can be.

A PT scheme for sweetpotato aims to provide growers with high-quality planting material that is free from pests and diseases, particularly viruses.

PT schemes will become vital as sweetpotato increases in importance as a world food crop. Already it is ranked eighth, and is now grown in more than 100 countries. Every year, around 125 million tonnes are produced, of which about half is for human consumption; the rest is used in animal feed (FAOSTAT 2007). China grows about 80% of the world's sweetpotato; Africa, Latin America and North America produce most of the remainder. In a number of Asian countries, sweetpotato is eaten as a substitute for rice and wheat, especially by the less well off. It is also important across the Pacific region, although only small amounts are grown relative to the global total. In parts of Melanesia, sweetpotato is the dominant staple.

In Papua New Guinea, about 2.9 million tonnes of sweetpotato are grown each year. In 2004, this was calculated to be worth A$700 million in terms of the cost of importing rice with an equivalent food energy (Bourke and Vlassak 2004). In Solomon Islands in the same year, production was estimated as 280,000 tonnes, 65% of the total staple food crops grown, with a value of A$42 million (Bourke et al. 2005). As well as providing energy, sweetpotato has useful quantities of provitamin A, ascorbic acid, riboflavin, iron, calcium and protein. Its overall value means that the demand for sweetpotato will grow as population increases.

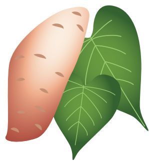
The risk of insect pest infestations and pathogen-related diseases will increase as more sweetpotato is grown. Sweetpotato is vegetatively propagated, so pests and diseases can spread from one generation to another. Among the insect pests, sweetpotato weevil (*Cylas formicarius*) is the most troublesome. Fungi, nematodes and viruses all cause diseases limiting production. Viruses and phytoplasmas are of particular concern: about 20 infecting sweetpotato have been reported worldwide (Loebenstein et al. 2009) and, during ACIAR project SMCN/2004/071 (*Reducing pest and disease impact on yield in selected Papua New Guinea sweetpotato production systems*), three previously unrecorded viruses in Papua New Guinea were recorded. On many occasions, infections with two or more viruses have been detected in the same plant.

In a number of countries, sweetpotato viruses are associated with significant yield decline (Loebenstein et al. 2009). So far, the search for a source of resistance has been unsuccessful, so the best solution is to produce planting material free from infection, and to make this easily available to growers, regardless of whether reinfection might later occur. This requires a number of different technologies: virus elimination, virus testing, rapid plant multiplication and making the plants available to growers as soon as possible.

Collections of the main commercial sweetpotato varieties grown in the Eastern Highlands and Western Highlands provinces of Papua New Guinea have now been made, tested for viruses and treated to eliminate infections. It is hoped that these healthy plants will show similar impressive early maturity and increased yield to those treated in the same way in Australia, China, the Philippines, South Africa and the United States.

In this manual, we describe, in simple terms, all the important stages of a modern PT scheme. We aim to demystify a process that can sound daunting, and encourage others to try these methods on sweetpotato and other important root and tuber crops.

Notes



Collecting, transporting and growing plants in pots

In this chapter, you will learn how to take vine cuttings and storage roots from the field, record vital information, transport the cuttings and roots back to the research facility, and take care of them. It is important to collect information carefully at this time, because you might want to use it later to compare what growers tell you about varieties with your own observations in field trials, especially after plants have been treated for virus infections.

What equipment do I need?

- Scissors, knife or scalpel
- Methylated spirits, laboratory-grade ethanol (70%) or isopropyl alcohol
- Household bleach (diluted to a concentration of 1.5%; see Appendix 1)
- Facial tissues, paper towels or newspaper
- Plastic bags
- Permanent marker pens
- A sturdy cardboard box in which to put plastic bags containing sweetpotato vines, and/or paper bags containing storage roots. For vines, an esky with ice or cold packs is ideal

When collecting plants from farmers' fields, vine cuttings or storage roots are both suitable, but note that:

- vine cuttings must be potted up within 3–4 days
- storage roots can be stored for longer periods—weeks or even months—before being planted in soil or potting mixes.

1.1 Information to collect: a note on passport data

Over the years, an international format has been devised for describing varieties of many crops and promoted by Bioversity International (previously the International Plant Genetic Resources Institute and, before that, the International Board for Plant Genetic Resources). The preface to the descriptor list for sweetpotato (CIP, AVRDC and IBPGR 1991) states,

'the descriptor list provides an international format and thereby produces a universally understood "language" for all plant genetic resources data'.

The purpose of the international format is to have a rapid, reliable and efficient means for storing, retrieving and communicating information, and to assist with sharing and use of the germplasm.

All crop descriptor manuals are divided into a number of sections. The one that interests us is the passport section,[1] which is used when collecting crop varieties. The passport section is divided into two parts: accession data and collection data. Accession data are mainly applied to plant specimens by curators at the time accessions enter into a collection (e.g. a gene bank or herbarium). Collection data document the location and date of collection, and the name of the person making the collection; they also describe the plant, accompanied by photographs and/or drawings made at the time of collecting. All this information provides a vital reference and should be collected carefully.

The amount of collection data varies, depending on the type of research being done or the purpose of the collection. At the very least, it is recommended that the data listed in the next section (Section 1.2) be collected.

1 The passport section is provided in Appendix 9; the full citation (CIP, AVRDC and IBPGR 1991) is in the references.

1.2 Collection data: information to record when collecting vines and storage roots

As a sample is collected, label it with a collection number. Then record the following information in a collection notebook:

- collection number—an identification number for the vine or storage root; if the sample is entered into a collection (e.g. a germplasm or herbarium), the curator will give it an accession number (a unique identifier, for that sample only)
- collector's name and institute
- donor's name—the farmer's name from whom the plant was collected
- variety name
- date collected
- location collected
  - village (with distance in kilometres from the nearest town) and province
  - GPS coordinates, if possible
- altitude (if using GPS)
- source of the collection
  - farm
  - market
  - institute
- type of sample
  - storage roots
  - vine cuttings.

For a herbarium sample:

- give the sample the same number as the collection number
- take a photograph of the sample, with the collection number
- state where the sample is kept.

Remember, it is very easy to make mistakes when collecting plants in the field, and a mistake here is difficult to change later. Label the plants carefully!

1.3 Collecting and preparing vines for transport

Scissors, knives and scalpel blades can be disinfected in the field by immersing them in methylated spirits, laboratory-grade ethanol (70%) or isopropyl alcohol for 10 minutes. Alternatively, they can be dipped for a few seconds in alcohol and then flamed (using a spirit lamp, candle or cigarette lighter) or placed in household bleach (diluted to a concentration of 1.5%) for 20 minutes. Note the following precautions:

- Be careful to shake off excess ethanol/alcohol before flaming! Allow instruments to cool before next use; if using scalpel blades, be sure to carry extra blades.
- Bleach will rust and shorten the useful life of scissors, knives and scalpel blades. Ethanol/alcohol is a more economical option if you do not want to replace your instruments every few days or weeks.

Look for plants in the garden that appear healthy. Avoid plants that have red or purple marks along the veins, ring spots between the veins, unusually small leaves, or twisted leaves and petioles with brown, dry scabby areas (Figure 1).

To collect vines, follow these procedures:

- Use disinfected scissors, knife or scalpel blade to cut vines approximately 30 cm long. Cut actively growing vines—that is, from young plants; these will grow faster, although any piece of vine will develop roots and shoots provided that it contains undamaged nodes.
- Between taking cuttings from different plants, wipe the scissors, knife or scalpel blade with paper towels soaked in

household bleach (diluted to a concentration of 1.5%; see Appendix 1), or dip in ethanol/alcohol and flame.

- Wrap the base of stems individually in damp facial tissues, paper towels or newspaper, and place all the cuttings of the same variety in a plastic bag.
- Label the bag on the outside with the collection number (see Section 1.2) using a permanent marker pen, and also place a tag, with the same number on it, inside the bag.
- Put the plastic bag in a cardboard box in the shade, or in an esky with ice or cool packs.

1.4 Collecting and preparing storage roots for transport

- Dig the storage roots from the field and brush off excess soil. Select roots that are free from obvious signs of pests and disease.
- Place the roots in a bag or box, and label it. Paper bags or cardboard boxes are best. If plastic bags are used, make holes in the bags to allow air flow, or the storage roots will rot.
- If the roots are to be stored for a short time (2–4 weeks), store them in a cool, dark place. In hot climates, you can use a domestic refrigerator or esky. But be careful: check the temperature of the refrigerator, and make sure it is not below 8 °C; if it is, chilling damage may occur, causing storage roots to rot.
- If roots are to be kept for several weeks to a few months before they are planted, store them in a cold room below 14.5 °C (above 15 °C, the roots will sprout) and at 85% humidity. Make sure that the temperature does not fall below 8 °C.

Do not wash roots, since washing allows entry of bacteria, which can cause rotting.

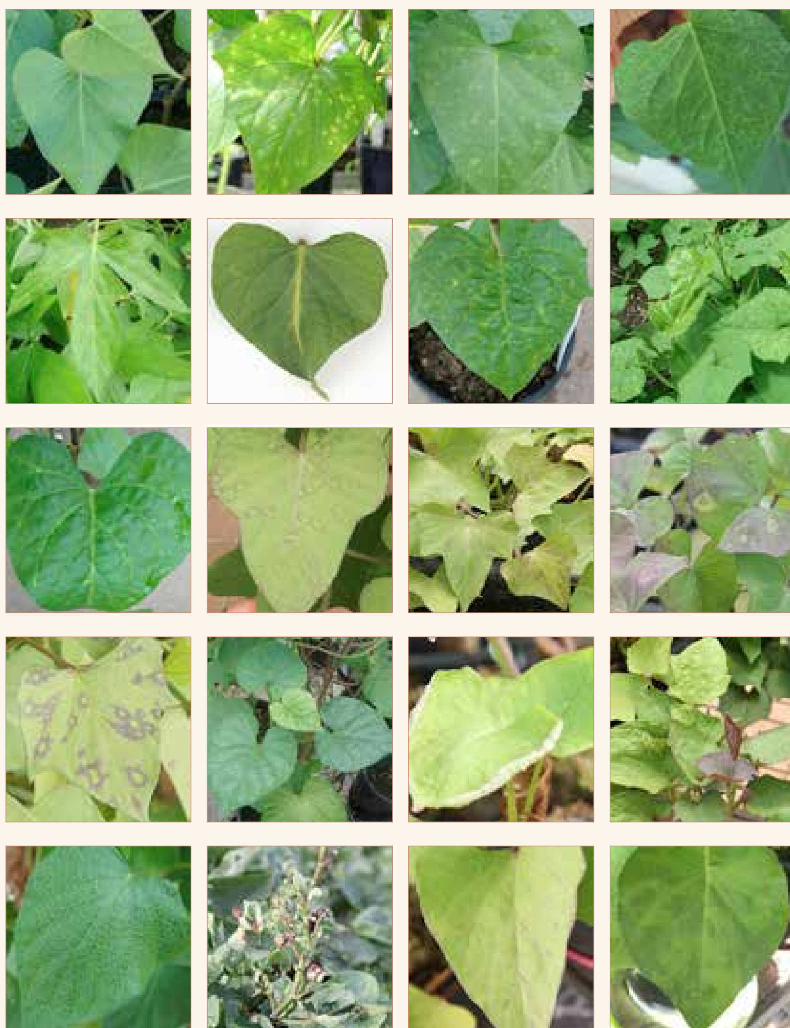


Figure 1
Plants to avoid when collecting samples. Note that virus, phytoplasma and fungal infections can cause many symptoms (yellow spots, ring spots, purple lines along the veins, curled leaves, little leaves and scabby leaves). Only the top left leaf is healthy.

1.5 Growing collections in pots

Once collections have been made and taken back to the research facility, vines and storage roots need to be grown in a screen house designed to minimise entry of insects that may be virus vectors, or in another insect-proof enclosure.

If you do not have an insect-proof screen house, grow the plants indoors, where there is sufficient light, or place them in a screened compartment on a bench. If light is not sufficient, place the plants under standard fluorescent lamps. The reason for growing the plants indoors is to prevent insects from colonising them and spreading virus infections. Also, new growth from plants grown this way has less fungal and bacterial contamination than plants taken directly from the field, and the shoots can be easily disinfected before they are grown in a sterile tissue culture medium.

What equipment do I need?

- Flower pots or polythene bags (15 cm in diameter)
- Commercial potting mix; or sterilised soil, chicken manure and river sand in the ratio of 3:2:1. In Australia, a pasteurised mix of sand, peat and perlite (or vermiculite) is used (Appendix 2)
- Household bleach (diluted to a concentration of 1.5% sodium hypochlorite; see Appendix 1)
- Watering can
- Plastic or aluminium labels or tags that can be tied around the stems of the sweetpotato vines
- Material for an insect-proof compartment, inside on a bench
- Personal protective equipment: gloves and mask when working with dry, dusty potting mixes (Appendix 3)

1.5.1 Vines

- Remove the last three leaves at the base of the vine.
- Rinse the vines under fast-flowing water from a tap to remove any insects that might be present—mealybugs, whiteflies and the larger species of mites.
- Place the vines in household bleach (diluted to 1.5% sodium hypochlorite) for 15–20 minutes.
- To remove the bleach, rinse the cuttings in distilled water, or boiled water that has been cooled.
- Insert the cut ends of two or three vines 4–6 cm into the potting mix or soil, in the pots or polythene bags.
- Water thoroughly, although the plants will not use much water until roots and new leaves develop. **Water from the bottom of the pot or polythene bag (see Section 1.6), to reduce contamination that might occur if soil particles and bacteria splash onto the vines and leaves.**
- Immediately put the plants in an insect-proof enclosure—that is, a screen house or glasshouse, or indoors in a screened compartment on a bench.
- Keep the plants on their own: do not keep them in the same screen house or glasshouse as healthy plants. If there is no alternative, keep them in a screened compartment.
- It is important to label pots correctly (Figure 2). The label should have:
  - the collection number (mandatory)—do not use the variety name because different varieties might have the same name
  - the date that the vines were potted up
  - where the vines were collected
  - the collector's name.
- Tie the label around the vine, or place it in the soil. **Use both methods (label it twice) if you want to keep the plant for some time**. Always check the collection number and the spelling of the variety—mistakes are often made at this potting stage!

- When the plants are growing, remove all but one vine per pot.
- Provide plants with fertiliser if they are kept in the pots for more than a month. If using Qmix (Appendix 2), there are sufficient nutrients for about 6 weeks. After that time, use a foliar fertiliser (e.g. Aquasol™ or Thrive®), and slow-release granules (e.g. Osmocote®). Refer to the product label for application rates.
- Check the vines at least twice a week to see that the soil has sufficient water, but do not overwater. Also check for insects and mites; where necessary, spray with an insecticide (e.g. a synthetic pyrethroid product) that is active against both. Fungal infections are unlikely; if they occur, they are best treated by removing the infected leaves or stems, rather than by fungicides.

1.5.2 Storage roots

- Wash or brush excess soil from the roots.
- Plant one or two roots per pot or polythene bag (Figure 3). Choose pots or bags that are appropriate to the size of the roots, and cover the roots with about 2 cm of commercial potting mix or sterilised soil (see Section 1.5.1). Water thoroughly.
- Immediately place the roots in an insect-proof enclosure—that is, a screen house or glasshouse, or indoors in a screened compartment on a bench.
- Keep the roots on their own: do not keep them in the same screen house or glasshouse as healthy plants. If there is no alternative, keep them in a screened compartment.
- Label the roots (see Section 1.5.1).
- When the roots develop vines, provide them with fertiliser (Section 1.5.1).

Check the vines two or three times per week to see that the soil has sufficient water, but do not overwater. Also check for insects and mites; where necessary, spray with an insecticide (e.g. a synthetic pyrethroid product) that is active against both. Fungal

infections are unlikely; if they occur, they are best treated by removing the infected leaves or stems, rather than by fungicides.

Keep the plants that you have grown from cuttings or storage roots away from all others, especially those you are distributing or using as part of the pathogen-testing scheme. The plants need to be grown in a separate insect-proof enclosure for at least 8 weeks, and checked for insect and mite infestations. Also, be careful not to overwater. Check at least twice a week.

Check the external walls of the screen house regularly for damage and holes, and repair as necessary. Keep the door closed, and keep entry to a minimum. See Appendix 4 for best practices for the screen house environment.

Figure 2
Newly planted vines maintained on a screened bench. Note the large label that is well secured to the stem of one of the cuttings.

Figure 3
Arrangement of roots in a pot before they are covered with soil or potting mix

1.6 A note on watering

Watering has been mentioned several times in this chapter. We have said to water thoroughly, check that the soil has sufficient water, avoid overwatering, and water from the bottom of the pot. What do all these mean?

Water is crucial to the health of the sweetpotato plants after they have been potted up, but there is no hard-and-fast rule on how much water to give them, or how often.

There are two ways of watering sweetpotato: from the top or from the bottom of the pots or bags.

If you are watering from the top, spray water over the leaves until the soil is saturated and the excess is draining from the bottom of the pots or bags. Preferably, have the pots or bags sitting on a wire-mesh bench so that the water escapes to the floor.

If you are watering from below to minimise soil splash onto the vines (see Section 1.5.1), stand the pots or bags in saucers or trays, and add 1–2 cm of water to the saucers or trays, allowing the soil to wet from the bottom up. Do not allow the pots or bags to stand in the water for more than an hour, or the soil will become waterlogged and the plants will suffer.

The difficult part is to decide when to water again. Here is where you need to do some checks:

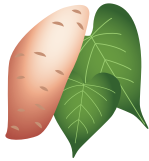
- Note the weight of the pots or bags before and after watering; when you come to water again, pick up a pot or bag and see if it feels light, as it was before watering.
- Look at the leaves to see if they have wilted since you last watered the plants.
- If the lower leaves are yellow and dying early, this can be a sign of either underwatering or overwatering.
- Put your finger into the soil to a depth of about 2–3 cm and check whether the soil feels wet, moist or dry.

If the pots or bags feel light and the foliage is limp or has wilted, water again. If the pots or bags are still heavy and the soil is wet, do not water.

The important points are as follows:

- Vine cuttings and plants deflasked from tissue culture do not have functioning roots, so water uptake will be low. Be especially careful not to overwater.
- Do not water plants of any age on a schedule; instead, check the condition of the plants and the soil, especially when it is hot in the screen house, and water (or not) based on your observations.

Notes



Establishing plants in tissue culture

You now have a supply of new growth from the plants that you collected in the field or from the market. The next task is to grow the plants in tissue culture, and to start the process of eliminating any virus infections that may be present.

While the plants are waiting to be established in tissue culture, they have to be kept in a screen house or indoor screened compartment, to prevent infestations of insects. They must also be provided with adequate nutrition for vigorous growth (see Section 1.5.1) and watered from the bottom (by adding water to a saucer or tray in which the pots or polythene bags are standing) to prevent soil splashing onto the shoots. Watering plants in this way reduces contamination of the shoots by bacteria and fungi and increases the chance that they can be successfully cultured in the laboratory.

You now need to grow the plants in a tissue culture medium, and to heat treat them when they are large enough to tolerate the process. Heat therapy of plants in tissue culture and pots is described in Chapter 3.

2.1 Collecting vines for tissue culture

What equipment do I need?

- Laboratory-grade ethanol (70%) or isopropyl alcohol
- Spirit lamp or candle, and matches
- Sharp scissors, or scalpel with blades
- New plastic bags, or clean bottles or beakers soaked in household bleach (diluted to a concentration of 1.5%; see Appendix 1) for 20 minutes and rinsed well
- Permanent marker pens and labels
- Personal protective equipment (Appendix 3)

After you have used instruments to take cuttings of vines of one variety, sterilise the instruments before taking cuttings of vines of a different variety.

Appendix 5 describes good laboratory practice; this includes maintaining a high standard of personal hygiene and laboratory cleanliness. Also see Appendix 3, which lists personal protective equipment. When working in a laboratory, a clean lab coat must always be worn. Gloves must also be worn, especially during tissue culture operations and virus indexing. There will also be occasions when a mask will be needed.

The procedures are as follows:

- In the screen house, look for healthy, young, vigorous shoots on plants that are no older than 6 weeks. Check that the nodes are green, not brown.
- Wearing gloves, and working with plants of one variety at a time, use scissors or a scalpel blade to cut off vines about 150 mm long, containing 3–8 nodes.
- Remove leaves from each vine, leaving only the first fully open leaf and the developing leaves. When removing the leaves, cut the petioles 5–6 mm from the stem.
- Place the vines of each variety in a clean plastic bag or bottle, and label it (Figure 4).
- Do not add water, damp tissues or paper towels to the bags or bottles, because this increases the risk of spreading contamination.
- Before repeating the procedure on another vine of the same variety, disinfect the scissors or scalpel blade by dipping in ethanol/alcohol for 5–10 seconds, flaming, and leaving to cool.

Figure 4
Vines cut from plants and placed in a beaker (left), and a plastic bag (right). Note the large labels.

- Repeat the same procedures on vines of each of the other varieties.
- If the screen house is far from the laboratory, or if the shoot tips need to be posted or transported to the laboratory, they can be sent in an express post bag or esky, together with a small ice pack. The ice pack should be wrapped in a paper towel to prevent it coming into direct contact with the cuttings. The cuttings need to reach the laboratory within 24 hours.

2.2 Preparing shoot tips and node cuttings for tissue culture

What equipment do I need?

- Laminar air-flow cabinet
- Sterilised forceps, and scalpel with blades
- Laboratory-grade ethanol (70%) or isopropyl alcohol
- Bacticinerator, bead steriliser, spirit lamp, Bunsen burner, or candle and matches (Figure 5)
- Sterile 150 mL bottles
- Sterile distilled water in 450–600 mL screw-capped bottles
- Beaker
- Tween 20® (a common detergent used in biology)
- Paper towels for swabbing benches and other surfaces with ethanol
- Plastic plant tags or sticky labels, and pencil
- Personal protective equipment (Appendix 3)

Double check that labels are correct; a mistake here will be difficult to correct later. Sterilise all the vines of one variety at one time.

Before using the laminar flow cabinet, it is important to put the equipment inside and then turn on the UV lights for 20–30 minutes. After this, wipe the inside of the cabinet with ethanol/alcohol. **Do not forget to turn off the UV lights before you start work!**

Appendix 5 describes best practice for tissue culture laboratories. It stresses the need to have a separate room for any material brought in from the outside, such as vine cuttings. Plant material should never be taken into the rooms used for tissue culture operations. Best practice also includes high standards of personal hygiene, as well as hygiene inside the building.

a. Glass-bead steriliser

b. Bacticinerator

Figure 5

Two methods of sterilising cutting equipment. Spirit lamps, Bunsen burners and candles are not recommended because they have naked flames that could lead to accidents in the confined space of the laminar flow cabinet, especially when ethanol/alcohol is present.

The equipment needed and the steps in preparing shoot tips and nodes for culture, illustrated in Procedure 1 on page 35, are as follows:

- Disinfect the scissors or scalpel blade by dipping in ethanol/alcohol and flaming.

- Rest the vine in a Petri dish, or on a tile or paper towel, and cut off the shoot tip, together with 100–150 mm of stem.
- Cut single nodes from the same vine, leaving 5–10 mm of stem above and below the bud.
- Remove any remaining petioles, cutting them about 5 mm from the stem.
- Between each vine, disinfect the scissors or scalpel blade by dipping in ethanol/alcohol and flaming; allow instruments to cool before continuing.
- Repeat with the shoot tips and nodes of the other varieties.
- Place a maximum of 10 shoot tips and/or trimmed nodes, of the same variety, in a clean, sterilised 150 mL bottle.
- Using a pencil, write the variety name on a thin plastic plant tag cut to the appropriate size and place inside the bottle. Alternatively, use a sticky label placed on the outside of the bottle.
- Turn on the laminar air-flow cabinet to begin sterilising the work area.
- Add 100 mL of household bleach (diluted to a concentration of 1.5%; see Appendix 1) and three or four drops of Tween 20 to the bottles, and then place the bottles on a shaker (or shake by hand) for 20 minutes.
- After shaking, place the bottles in the now sterile laminar air-flow cabinet.
- In the laminar flow cabinet, discard the bleach, and rinse the shoot tips and nodes three times in sterile distilled water to remove traces of bleach.
- Remove the discarded bleach and sterile water from the laminar flow cabinet, and wipe down the interior of the cabinet with ethanol/alcohol. Also wipe with ethanol/alcohol the outside of the bottles containing the shoot tips and nodes.

Use a pencil when writing on tags because pencil does not come off in bleach.

Procedure 1 Equipment and methods to sterilise shoot tips and nodes

a. Trimmed vines (about 150 mm long) from the screen house, with 5 or 6 nodes each

b. Cutting off the shoot tip in a Petri dish

c. Vine pieces uncut, and cut into shoot tips and nodes

d. Vines cut and uncut

e. Vine pieces in 150 mL bottles ready for bleach treatment. Note the maximum of 10 in each bottle

f. Adding the bleach (1.5% sodium hypochlorite)

g. Bleach added, and a label

h. Shaking the bottles for 20 minutes

i. Rinsing the cuttings after the bleach has been removed

2.3 Placing shoot tips and node cuttings in tissue culture

What equipment do I need?

- Laminar air-flow cabinet
- Sterile Petri dish, tile or sterilised paper towel on which to cut the shoot tips and nodes
- Bacticinerator, bead steriliser, spirit lamp, Bunsen burner, or candle and matches (Figure 5)
- Laboratory-grade ethanol (70%) or isopropyl alcohol
- Sterilised forceps, and scalpel with blades
- Paper towel for swabbing benches and other surfaces with ethanol/alcohol
- Jars of culture medium approximately 50 mm in diameter and 130 mm high, containing multiplication medium (Appendix 6)
- Small light bank or growth room to grow plants in vitro
- Labels and pencil

It is a good idea to have at least three sets of instruments (forceps and scalpel with blades) when working in the laminar flow cabinet: one set cooling (after being sterilised in a bacticinerator or bead steriliser, or after being flamed), one set soaking in ethanol/alcohol before sterilisation, and one set in use.

When using the laminar air-flow cabinet, be careful to maintain a free flow of air over the work surface—that is, do not put bottles or other equipment in the way of the air from the filter.

Appendix 6 gives the recipes for multiplication and shoot/node initiation culture media, as used at Redlands Research Facility. Appendix 7 illustrates the procedures for making culture media.

The steps in placing shoot tips and nodes in tissue culture are illustrated in Procedure 2 (page 39).

- Working with one bottle at a time, remove the shoot tips and nodes, and place them in a sterile Petri dish, on a tile or on a paper towel.
- With a sterilised scalpel blade, trim the stems 1–2 mm above and 3–5 mm below each node. Trim off the remains of the petiole, and remove all tissue damaged during sterilisation—the tissue will have changed colour from green to a translucent, pale, brownish green. (If not removed, it may become a source of contamination for the developing plant.)
- Place the nodes and the shoot tips in the multiplication medium. Make sure that the node is the right way up—that is, the same orientation as on the vine. Put no more than five shoot tips or nodes in a bottle.
- Repeat these procedures for the shoot tips and nodes of each variety, wiping down the laminar flow surface, and sterilising the instruments and cutting surfaces with ethanol/alcohol, each time a new variety is treated. If using a paper towel, discard it and use another that has been sterilised.

Label each culture vessel with:

- collection number and variety
- date of initiation
- initials of scientist or technician
- total number of plants initiated per variety
- date for subculturing (at intervals of approximately 12–16 weeks).

Another method of labelling is to use coloured plastic beads for the different stages of the pathogen-testing process—for example, one colour might be used for cultures ready to undergo heat therapy, another for the sweetpotato field collection, and a third for plants regrown from meristems to be indexed (checked for viruses) on *Ipomoea setosa* (see Chapter 5). If colour codes are used, it is important that a master sheet explaining the codes is easily accessible for everyone to refer to.

2.4 Incubating shoot tips and node cuttings in the laboratory

- Incubate the cultures on a bench, on shelves or in a growth room at 25 ± 2 °C under lights of 5,000–10,000 lux. The following setup has been found to be satisfactory:
  - six fluorescent lights: a mixture of three 'grow' lights (at least one blue and one red) and three white lights, providing a light intensity of about 5,000–10,000 lux
  - a day/night setting of 16 hours light and 8 hours dark
  - lights placed vertically to give the plants even lighting, or lights placed horizontally above each of the shelves (Procedure 2, page 39).
- Look at the cultures after 5 days, then regularly check for contamination:
  - If present, bacterial contamination may be seen after a few days. Colonies of bacteria often form at the base of the stems inside the culture medium. They appear as opaque haloes that develop rapidly into a mass of various colours, often orange–pink, green–black or black.
  - Although fungal contamination may be seen after a few days, it usually is not seen for a week or so. It appears as furry, fuzzy colonies with no clearly defined edges growing on the surface of the culture medium (Figure 6).

Procedure 2 Preparing shoot tips and nodes for tissue culture

a. Laminar flow cabinet

b. Sterilised scalpels and forceps ready for use

c. Sterilisation using a bead steriliser

d. Trimming to remove the bleached ends of the stems

e. Nodes in tissue culture—note that there are only four

f. Four nodes in a labelled bottle

g. Tissue cultures incubated under a mixture of 'grow' and fluorescent lights

h. A simple metal-framed bench with fluorescent lights at the top connected to a timer. Note: unless lights are placed above each shelf, the lower shelves receive less light

- Discard any cultures that are contaminated; do not try to save these by trying to remove the infection with bleach or fungicide.
- When putting lids on vessels, screw them on firmly and then turn them back a quarter turn. This will allow gas exchange with the outside, and prevent the build-up of ethylene, which is produced by the plantlets. Ethylene build-up can be lethal to plants.

! When the lights go off at night, temperatures cool slightly, and culture vessels and lids shrink; when lights come on during the day, culture vessels and lids swell. As a result of these changes, air enters the culture vessels. The culture room, benches or shelves where the cultures are kept must therefore be kept as clean as possible. Access to the culture room should be limited to essential inspections, to reduce the chance of contamination.

If contamination becomes a problem, cover the top of the bottles in GLAD®Wrap; this will allow gas exchange, but exclude fungal spores.

Figure 6
Contaminated cultures showing black-coloured fungi and orange-coloured bacterial infections (left), and healthy cultures (right). Note that the latter have different coloured labels, depending on their use.

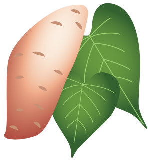
2.5 Subculturing plants in the laboratory

Sweetpotato plants can be maintained in tissue culture for at least 2 months before they need to be subcultured—that is, transferred to new culture medium. During subculturing, the plants are multiplied. Leaves and roots are removed, and each stem is cut into a shoot tip and several nodes, in the same way that the original vine was treated. The pieces are then transferred to culture medium of the same composition as the original plants (multiplication medium) (Appendix 6).

If the plants are to be heat treated (see Chapter 3), put one piece in each bottle. If the plants are to be used for general maintenance or multiplication, place four pieces (shoot tips or nodes) in each bottle.

Plants can be maintained in this way indefinitely. Usually, when they are required for heat therapy, they are used when they are 4–6 weeks old.

Notes



Heat therapy

There are two ways of heating sweetpotato plants to free them from virus infection: heating the plants growing in pots, and heating sterile plants growing in tissue culture. Both methods have advantages and disadvantages. In this section, we describe the techniques, using the plants that were established in pots and tissue culture in Chapters 1 and 2, respectively.

First, what does therapy mean? A therapy is a treatment given to heal a disorder. In our case, heat is the treatment, and the disorder is the disease that results from infection by internally borne viruses or virus-like micro-organisms. 'Heat treatment' is another term that could be used, but 'heat therapy' is the accepted name used by scientists.

Important assumptions underlie heat therapy:

- The plants are already infected by viruses and/or other internally borne micro-organisms.
- Sweetpotato plants are more tolerant to heat than the micro-organisms within them.
- The plants can be regrown after treatment.

The pot method is used for many plant species, since it is relatively straightforward. Actively growing plants are placed in a cabinet, and the temperature inside is increased by stages over several weeks. The meristems are then removed aseptically from the shoot tips, and placed in a sterile tissue culture medium. The meristem is at the tip of the shoot; it is composed of actively dividing cells that form the tissues that become the leaves and stems.

There are a number of reasons for using the meristems. One is that the meristem is often free from viruses; it is without vascular tissues, making it difficult for viruses to infect the cells there. Another is that the heat accelerates the growth of the plant, and at the same time reduces multiplication of viruses; this increases the chance that some meristems will develop free from virus, and can then be regrown into healthy plants.

The tissue culture method is similar to the pot method, except that the plants are heat treated while growing in a sterile medium. It has the obvious advantage that the shoot tips do not need to be sterilised. Another advantage, although less important, is that tissue culture bottles are smaller than pots, and so more can be placed in the cabinets used for heat therapy. However, a disadvantage with this method is that the plants in tissue culture are much smaller than those grown in pots; consequently, the meristems are also smaller, and this reduces their chance of survival when they are cut out and placed on the culture medium.

The two methods, pot and tissue culture, are illustrated in Figure 7.

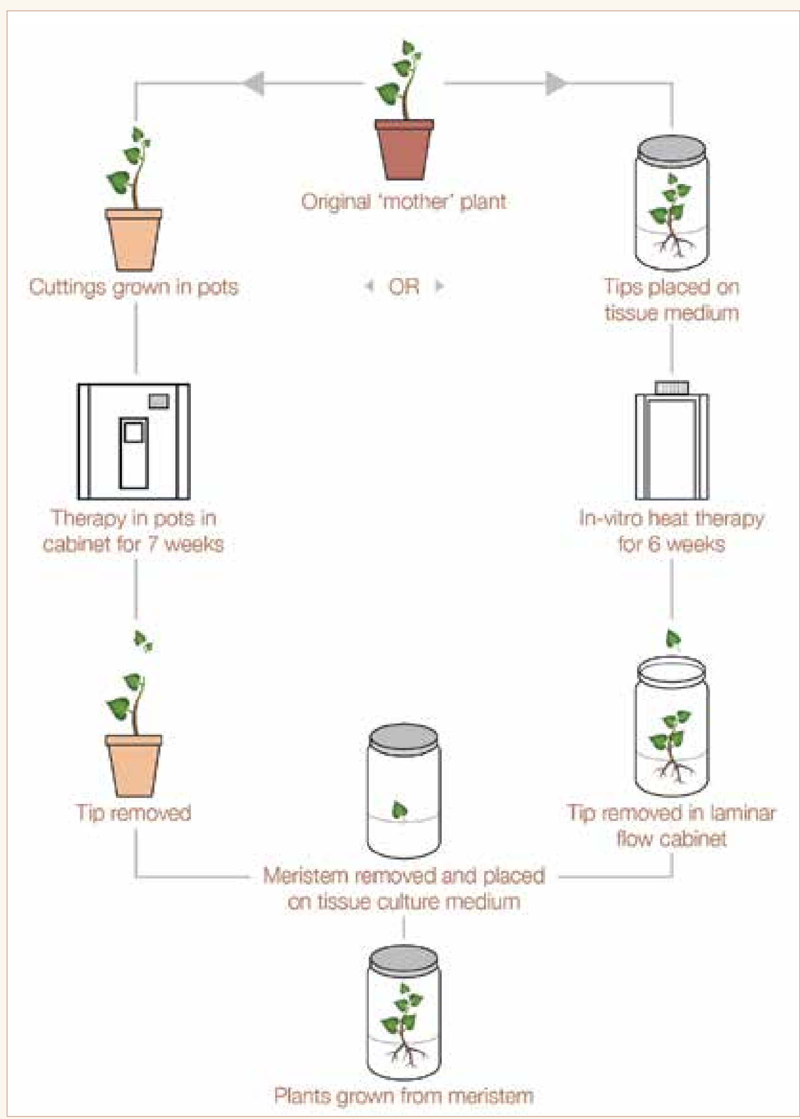


Figure 7
Heat therapy for sweetpotato plants grown in pots or in tissue culture, including subsequent removal of the meristems and their transfer to a tissue culture medium to regrow the plants

3.1 Pot method

What equipment do I need?

- A growth cabinet (hot box), capable of maintaining temperatures up to 40 °C, containing banks of lights—both fluorescent and incandescent—giving a light intensity of approximately 5,000–10,000 lux (Figure 8). The cabinet should have time controls for day/night periods of 16 hours light and 8 hours darkness
- Three or more thermometers
- Scissors, knife or scalpel blades

The effect of the temperature regime on sweetpotato plants is severe, although some varieties can withstand heat better than others. At the highest temperatures, plants may die. The aim is to use the correct amount of heat to treat the plants but not so much that they die. This is not always easy.

Heat therapy for plants in pots is as follows:

- Grow 3–5 plants of each variety, from vine cuttings, for 6–8 weeks. Usually 10–15 cm diameter pots are used because they fit well in many growth cabinets. Each pot has one plant. (Refer to Section 1.5.)
- When plants are 6–8 weeks old, place them in the growth cabinet. They will remain there for 7 weeks.
- Place thermometers inside the cabinet to check the temperatures in different areas of the cabinet. Cabinets often have 'hot spots'.
- Place plants of the same variety in different parts of the cabinet. If there are hot spots, this will prevent all the plants of one variety suffering from temperatures that are too high.

Figure 8
Cabinet used for heat treating potted plants. This is a commercial, purpose-built growth cabinet, which is more expensive than a converted refrigerator.

- Place the pots in saucers or trays. Water the plants from below by putting water into the containers. This avoids splashing bacteria and fungi from the soil or potting mix onto the vines.
- Grow the potted plants under the following temperature regime
    - 25 ºC for 7 days, followed by
    - 29 ºC for 14 days, followed by
    - 39 ºC for 28 days.

- During the first 2 weeks in the cabinet, trim the plants by removing growing tips with sterile scissors, knife or scalpel blade. This will encourage the plant to produce multiple shoots, increasing the number of meristems that can be taken and grown in tissue culture.
- During the 28 days when the plants are at 39 °C, it is important not to overwater, because waterlogged plants tend to die quickly at the highest (39 °C) temperature. Plants need to be checked daily, and the soil or potting mix should be allowed to dry before more water is added. Aim to apply water about every 2 days. If testing the soil with your finger (see Section 1.6) shows that it is still wet 48 hours after the last watering, let it dry and reduce the amount of water given to each pot when re-watering.
- At the end of 7 weeks, remove the new shoot tips and cut out the meristems (see Chapter 4). The new shoots are those that grew in the cabinet after the plants were trimmed. The appearance of the plants at this time is shown in Figure 9.

3.2 Tissue culture method

What equipment do I need?

- A growth cabinet (hot box), capable of maintaining temperatures up to 40 °C, containing banks of lights—both fluorescent and incandescent—giving a light intensity of approximately 5,000–10,000 lux (Figure 10). The cabinet should have time controls for day/night periods of 16 hours light and 8 hours darkness
- Three or more thermometers

a. Week 1 at 25 °C

b. Trimming the vines at 10 days

c. Week 2 at 29 °C

d. Week 4 at 39 °C

e. Week 5 at 39 °C

f. Week 6 at 39 °C

Figure 9
Appearance of potted plants after 1–6 weeks at three incremental temperatures

Heat therapy for plants in tissue culture is as follows:

- Grow 10 plantlets of each variety in tissue culture for 4 weeks. By this time, they should be growing vigorously, approximately half-way to the top of their containers.
- Place thermometers inside the cabinet to check the temperature in different areas. Cabinets often have ‘hot spots’.
- Place plants of the same variety in different parts of the cabinet. If there are hot spots, this will prevent all the plants of one variety from suffering from temperatures that are too high.
- Place the plants in the growth cabinet or incubator (Figure 10) for 6 weeks.

a. A converted refrigerator, which is factory fitted with four fluorescent lights built into the door, a heater, and a control panel to regulate heating and cooling, and to set times for the lights to turn on and off

b. Plants growing on a tissue culture medium in the converted refrigerator (at the beginning of heat therapy)

c. A closer view of the plants in culture

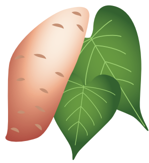
d. A purpose-built growth cabinet

Figure 10
Two types of growth cabinet: a converted refrigerator (a–c) and a commercial cabinet (d)

- Grow the plants under the following temperature regime
  - 29 °C for 7 days, followed by
  - 34 °C for 7 days, followed by
  - 37.5 °C for 28 days.
- At the end of the 6 weeks, remove the shoot tips and extract the meristems (see Chapter 4). The appearance of the plants at this time is shown in Figure 11.

Figure 11
The appearance of plants in tissue culture before (left) and after (right) heat therapy for 6 weeks.

Notes



Meristem culture

When heat therapy is complete, the next task is to remove the meristems at the tips of the vines, and place them in sterilised tissue culture medium under lights in the laboratory. There, they will grow into new plants.

Plants regrown this way may be free from viruses, but there is no guarantee. The success rate depends on the types of viruses that are present, and the skill of the person removing the meristems. Although it may seem difficult at first to cut out the meristems, because they are so small, you will improve with practice!

You only need one plant of each variety that is virus free. Fortunately, sweetpotato has a high multiplication rate in tissue culture, in pots in the screen house or in plots in the field, so even one plant can be made into many in a very short time. However, to get that one healthy plant, you need several plants to test.

Once the plants have regrown from meristems, the next task is to grow them in pots in the screen house, glasshouse or screened compartment, and to test them for known viruses—this is covered in subsequent chapters.

Figure 12 shows the different parts of the terminal (apical) bud. The sequence of events in removing the meristem under the microscope is illustrated in Procedure 3 (page 63).

a. Apical tip intact

b. Apical tip with leaflets removed

c. Apical tip with leaflets and lead primordia removed, exposing the meristematic dome

Figure 12
The terminal shoot of sweetpotato, and the steps from the removal of leaves around the meristem (left and centre) until it is exposed (right), so that it can be cut out and cultured on sterile tissue culture medium. Note that the meristem of the axillary bud at the node is similar to that of the terminal shoot.

4.1 Culture of meristems from heat-treated potted plants

4.1.1 Collecting the vines

What equipment do I need?

- Laboratory-grade ethanol (70%) or isopropyl alcohol
- Spirit lamp or candle, and matches
- Sharp scissors, or scalpel with blades
- New plastic bags, or clean bottles soaked in household bleach (diluted to a concentration of 1.5%; see Appendix 1) for 20 minutes and rinsed well in sterile distilled water
- Permanent marker pens
- Personal protective equipment (Appendix 3)

Do not forget to sterilise the instruments between taking vines from different plants.

The steps in collecting vines from heat-treated plants are as follows:

- In the high-temperature cabinet, choose a potted plant of one variety, and do the following
    - Disinfect the scissors or scalpel blade by dipping in ethanol/alcohol and flaming; allow to cool before using.
    - Wearing gloves, and using scissors or a scalpel blade, cut off vines about 150 mm long.
    - Remove most of the petioles, cutting them 5–6 mm from the stem.
    - Place the vines in a plastic bag or bottle, and label it (Figure 13).

   - Do not add water, facial tissues or paper towels to the bags or bottles because this increases the risk of spreading contamination.
   - Repeat the same procedures on the other potted plants of the same variety, and then all other varieties, in the cabinet. Continue to ensure that scissors or scalpels are disinfected between plants.

- If the screen house is distant from the laboratory, or if the vines need to be posted or transported to the laboratory, they can be sent in an express post bag or esky, together with a small ice pack. The ice pack should be wrapped in a paper towel to prevent it coming into direct contact with the vines. The vines need to reach the laboratory within 24 hours.

Figure 13
Taking vines for meristem culture: vines cut from plants after heat therapy (left); vines in a well-labelled plastic bag (right)

4.1.2 Preparing shoot tips and nodes

What equipment do I need?

- Laminar air-flow cabinet
- Laboratory-grade ethanol (70%) or isopropyl alcohol
- Spirit lamp or candle, and matches
- Household bleach (diluted to a concentration of 1.5%; see Appendix 1)
- Tween 20 (or other detergent)
- Sterile 100 mL bottles
- Sterile distilled water in 450–600 mL screw-capped bottles
- Paper towels for swabbing benches and other surfaces with alcohol
- Sterile Petri dishes, small tile or paper towels, for use as a cutting surface
- Sterilised forceps, and scalpel with blades
- Sticky labels and pen, or thin plastic plant tags and pencil
- Personal protective equipment (Appendix 3)

The steps, which are similar to those in Section 2.2 and are illustrated in Procedure 1 (page 35), are as follows:

- In the laboratory, sterilise the internal surfaces of the laminar flow cabinet with ethanol/alcohol, and sterilise all equipment to be used.
- Turn on the UV light of the laminar flow cabinet 20 minutes before use.

- Meanwhile, on the laboratory bench, and preferably in a different room from where the laminar flow cabinet is kept, prepare each terminal and node cutting
    - Disinfect scissors or scalpel blade by dipping in ethanol/alcohol and flaming; allow to cool.
    - Cut the vines from a heat-treated plant into terminal and single node cuttings, leaving 5–10 mm of stem above and below the bud.
    - Remove any remaining petioles, cutting them about 5 mm from the stem.
    - Place the trimmed terminal and node cuttings from the same variety into a clean, sterilised 100 mL bottle. Do not put more than 6 or 7 pieces in a single bottle.
    - Using a pencil, write the variety name on a thin plastic plant tag cut to the appropriate size and place it inside the bottle. Alternatively, use a sticky label placed on the outside of the bottle.
- Disinfect scissors or scalpel blade by dipping in ethanol/alcohol and flaming; allow to cool.
- Repeat the same procedures on the other vines of the same variety, and then on other varieties.
- Sterilise the terminal and node cuttings by one of the following methods (the first method is preferred)
    - Add ethanol/alcohol to cover the shoot tips and nodes for 1–2 minutes, remove the cuttings, rinse them thoroughly with sterile distilled water, then soak them in household bleach (diluted to a concentration of 1.5%; see Appendix 1) with three or four drops of Tween 20 (or other detergent) per 100 mL, and place the bottles on a shaker (or shake by hand) for 20 minutes.
    - Add household bleach (diluted to a concentration of 1.5%; see Appendix 1) with three or four drops of Tween 20 (or other detergent) per 100 mL to cover the shoot tip and nodes in the 100 mL bottles, and place the bottles on a shaker (or shake by hand) for 20 minutes.

- After sterilising, in the laminar flow cabinet, discard the bleach, and rinse the terminal and node cuttings three times in sterile distilled water. Remove the bottles with discarded bleach and sterile water, wipe down the laminar flow cabinet, and prepare a sterilised area for the next step.

4.1.3 Cutting out the meristems

What equipment do I need?

- Laminar air-flow cabinet
- Laboratory-grade ethanol (70%) or isopropyl alcohol
- Paper towel for swabbing benches and other surfaces with ethanol/alcohol
- Microscope with at least 40× magnification and lamp, if the microscope does not have built-in illumination
- Sterile Petri dishes, small tile or paper towel, for use as a cutting surface on the microscope
- Sterilised forceps, and scalpel with blades
- New hypodermic needles, eye scalpels or microtool scalpels
- Bacticinerator, bead steriliser, spirit lamp, Bunsen burner or candle
- Small 30 mL tubes of meristem medium (Appendix 6), prepared at least a day before meristem extraction (to ensure that the medium has set properly)
- Plastic plant tags or sticky labels, and pencil
- Personal protective equipment (Appendix 3)

In this manual, we suggest that you may use the buds at the nodes. Not everyone does this because there is a greater chance of virus infection than when using terminal buds; some protocols do use the buds at nodes, but only those close (no more than 25 mm) to the shoot tip. It is also time consuming to remove the meristems from buds at the nodes. However, these buds are useful when only a few terminal buds survive the heat treatment.

The equipment and procedures for cutting out the meristems are illustrated in Figure 14 and Procedure 3 (page 63), respectively. The methods are similar for plants that have been heat treated in pots and those growing in a tissue culture medium.

The procedures are as follows:

- Thoroughly clean and sterilise all equipment
  - Wipe the surfaces of the microscope and all equipment with ethanol/alcohol.
  - Wipe the inside of the laminar flow cabinet with ethanol/alcohol.
  - Place all equipment, including the microscope, in the laminar flow cabinet under a UV light for 20 minutes.
- Wipe the outside of the containers containing the plantlets with ethanol/alcohol before placing them in the laminar flow cabinet.
- Working with one bottle containing the shoot tips and nodes of one variety, transfer the shoot tips and nodes to a sterile Petri dish.
- Cut out the meristems from the shoot tips
  - Do this as quickly as possible, to avoid having the meristems dry out under the heat of the microscope lamp. If possible, use fibre optic 'cold light' illumination.
  - Use a scalpel blade to slice away the leaves around the terminal bud, leaving all but the last two or three leaf primordia surrounding the meristem.

- Use a sterile hypodermic needle to cut off the leaf primordia, until the meristematic dome is seen; this appears as a shiny, opaque area at the tip of the shoot, surrounded by two or three leaf primordia.
- Cut out the meristem dome using the hypodermic needle, and transfer it to the surface of the meristem growth medium (Figure 15).
- Repeat the same procedures for other bottles, making sure each time that you use sterilised instruments and cutting surfaces, and wipe down the inside of the laminar flow cabinet with ethanol/alcohol.

- Label each culture vessel with the
  - collection number
  - date of culture
  - initials of the person establishing the culture
  - total number of cultures for each variety
  - date for subculturing (at intervals of approximately 8–12 weeks).
- Incubate the cultures as described in Section 2.4.
- Monitor the cultures
  - Look at the cultures after 5 days, then check regularly for contamination.
  - If present, bacterial contamination may be seen after a few days. Colonies of bacteria often form at the base of the stems inside the culture medium. They appear as opaque haloes that develop rapidly into a mass of various colours, often orange–pink, green–black or black. Although fungal contamination may be seen after a few days, it usually is not seen for a week or so. It appears as furry, fuzzy colonies with no clearly defined edges growing on the surface of the culture medium (Figure 6).
  - Discard any cultures that are contaminated.
- Continue the incubation of cultures that are free from contamination. The meristems will take 8–12 weeks to develop into a plant.

a. Inside the laminar flow cabinet: stereomicroscope, forceps, mounted needles and scalpels

b. Instruments in alcohol, sterile hypodermic needles in plastic covers, and medium in 30 mL plastic bottles ready to receive meristems

c. Packet of hypodermic needles used to excise meristems

d. Hypodermic needles mounted on long handles

e. Bottles containing sterilised terminal and node cuttings, and culture medium. Note that the bottles are blocking the sterile air flow over the work surface and should be moved out of the way

f. Shoot tips ready to be dissected under a stereomicroscope

Figure 14
Equipment for meristem extraction

Procedure 3 Cutting out the meristem under a stereomicroscope

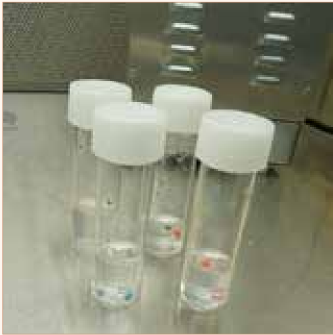
a. Using a scalpel to remove the young leaves

b. Using a hypodermic needle to remove ever-smaller leaf primordia surrounding the meristem

c. The meristem

d. The meristem removed

e. An axillary bud at a node



a. 30 mL tubes containing tissue culture medium

b. Meristem on medium in labelled bottle, but too small to see!

c. Shoot emerging from callus grown from a meristem; the red and blue beads indicate the type of media used and that the shoot has been heat treated

d. Stages in the development of plants from meristems

Figure 15
Meristems regrown

4.2 Culture of meristems from heat-treated plants in tissue culture

4.2.1 Cutting out the meristems

What equipment do I need?

- Laminar air-flow cabinet
- Laboratory-grade ethanol (70%) or isopropyl alcohol
- Paper towel for swabbing benches and other surfaces with ethanol/alcohol
- Microscope with at least 40× magnification and lamp, if the microscope does not have built-in illumination
- Sterile Petri dishes, small tile or paper towel, for use as a cutting surface on the microscope
- Sterilised forceps, and scalpel with blades
- New hypodermic needles, eye scalpels or microtool scalpels
- Bacticinerator, bead steriliser, spirit lamp, Bunsen burner or candle
- Small 30 mL tubes of meristem medium (Appendix 6), prepared at least a day before meristem extraction (to ensure that the medium has set properly)
- Plastic plant tags or sticky labels, and pencil
- Personal protective equipment (Appendix 3)

The meristems from heat-treated tissue culture plants are much smaller than those from heat-treated potted plants, and often die. Project experience has shown that survival of the meristems taken from tissue culture plants improves considerably if one leaf primordium is left when the meristem is cut out. The problem is that if a leaf primordium is left, there is a greater chance that

plants will still be infected by virus. **Note: This procedure is only for tissue culture meristems and must not be done when using potted plants for meristems.**

Be careful to maintain a free flow of air over the plant material—make sure that bottles and other equipment are not blocking the movement of sterile air over the work surface where the meristem is being extracted.

Handles for the hypodermic needles can be made from children's plastic paint brushes with the bristles removed; the handles should be soaked in ethanol/alcohol before use.

The steps in cutting out the meristem, illustrated in Figure 14 and Procedure 3 (page 63), are as follows:

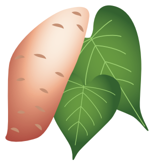
- Thoroughly clean and sterilise all equipment (see Section 4.1.3).
- Remove the shoot tips (approximately 10 mm) from the plants using a sterilised scalpel blade, and place them in a sterile Petri dish. Since the constant air flow in the laminar flow cabinet will rapidly dry out the shoot tips, it is best to have only three at a time in the Petri dish.
- **Do not** discard the plants in tissue culture from which the shoot tips have been taken. Place the containers back on the shelves of the growth room. If the meristems do not survive for some reason, or are found to contain virus, these plants can be used again.
- Remove the meristems from the shoot tips
  - Do this as quickly as possible, to avoid having the meristems dry out under the heat of the microscope lamp. If possible, use fibre optic 'cold light' illumination.
  - Use a scalpel blade to slice away the leaves around the terminal bud, leaving all but the last two or three leaf primordia surrounding the meristem.
  - Use a sterile hypodermic needle to cut off the leaf primordia, until the meristematic dome is seen; this appears as a shiny, opaque dome.

- Cut out the meristem, which might include the youngest leaf primordium, using a new sterile needle, and transfer it to the surface of the meristem medium (Figure 15).

- Repeat the same procedures for each tip, making sure that you use sterilised instruments and cutting surfaces, and wipe down the inside of the laminar flow cabinet with ethanol/alcohol after 2 hours work.
- Label the culture vessel with the
  - collection number
  - date of culture
  - variety name
  - initials of the person establishing the culture
  - total number of cultures for each variety
  - date for subculturing (at intervals of approximately 8–12 weeks).
- Incubate the cultures as described in Section 2.4.
- Monitor the cultures
  - Look at the cultures after 5 days, then check regularly for contamination. If present, bacterial contamination may be seen after a few days. Colonies of bacteria often form at the base of the stems inside the culture medium. They appear as opaque haloes that develop rapidly into a mass of various colours, often orange–pink, green–black or black.

    Although fungal contamination may be seen after a few days, it usually is not seen for a week or so. It appears as furry, fuzzy colonies with no clearly defined edges growing on the surface of the culture medium (Figure 6).
  - Discard any cultures that are contaminated.
- Continue the incubation of cultures that are free from contamination. The meristems will take 8–12 weeks to develop into a plant.
- Discard meristems that have not developed into shoots by 12 weeks, but have developed into a callus.

Notes



Tests for viruses: indicator plants

Although the plants have now been heat treated and regrown from meristems (or shoot tips), they still have to be checked for viruses. We cannot assume that all the viruses have been eliminated. This checking process is known as virus indexing or pathogen testing. The plants are often referred to as being pathogen tested (PT).

Although the checks are stringent, it is never possible to be certain that a variety is virus free. Viruses may be present that are undetectable by current testing methods. For example, symptoms may not appear on the indicator plant (*Ipomoea setosa*), or biochemical tests may not detect a particular virus in plant sap, either because the virus is at low concentration or chemicals present interfered with its detection. As well, biochemical and molecular tests (ELISA—enzyme-linked immunosorbent assay, and PCR—polymerase

chain reaction) test only for known viruses. If a virus is present that is new to science, these methods will not find it.

For these reasons, we can only say that a variety has been pathogen tested, and specify the methods used. We cannot say for certain that the variety is free from virus. Because of this, quarantine officers require that plants from other countries are grown in a secure facility for a period of weeks or months before they are released. During the time in quarantine, the plants are checked. These checks might be just visual, or, as happens for sweetpotato entering some countries, government regulations might insist that the plants go through a virus removal process again. This happens even if they were derived from plants that were heat treated, grown from meristems, and tested by ELISA or PCR elsewhere. After the virus removal process, they are tested again for viruses.

It is clear that pathogen testing is necessary for plants coming into a country; no country wants to import diseases with plant introductions. But pathogen testing is also necessary for plants used in any scheme that provides plants to growers, whether the plants are from other countries or are local varieties. It is essential that the plants are thoroughly tested by the most sensitive methods available, because the success of the scheme depends on the health of the material given to the growers. Experience from many countries tells us that sweetpotato plants without viruses produce better crops than those that are infected with viruses. The storage roots mature earlier, they are a better shape, and yields are higher.

There are several ways that plants can be tested for viruses. Most tests are complementary—that is, they support but do not duplicate each other, giving us greater confidence that the results are correct. The methods most often used for testing are:

- electron microscopy: uses high magnification to look for viruses in the sap

- ELISA: a biochemical method based on serology, using antibodies from blood serum to detect viruses
- mechanical inoculation: rubbing sap onto sensitive indicator plants
- graft inoculation: grafting a stem or shoot onto an indicator plant; for sweetpotato, the indicator plant is *I. setosa*, a species that is highly sensitive to many, perhaps all, viruses (as well as phytoplasmas) that infect sweetpotato, and readily shows symptoms of infection
- PCR: uses pieces of nucleic acid called 'probes' that are made to detect a particular virus; for each virus, there is a different probe.

All these methods have both advantages and disadvantages. For example, electron microscopes are expensive, and this method also requires that the viruses are in relatively high concentration in plant sap so they can be seen easily; electron microscopy is now not considered sensitive enough to be used on its own. More sensitive serological and molecular methods (ELISA and PCR, respectively) are used to provide confidence that the plants being tested are without detectable infections. These methods are often used in conjunction with indicator plants, such as *I. setosa*.

ELISA detects proteins on the coat of virus particles, whereas PCR detects the core genetic material. Of the two, PCR is more sensitive; it is more sophisticated and uses complex (and expensive) equipment. Another PCR process, real-time PCR (qPCR) is 1,000 times more accurate than conventional PCR. The advantage of ELISA, apart from its relative simplicity, is that kits are available for the detection of many viruses, including those infecting sweetpotato. These can be used by laboratory technicians with, perhaps, little training in virology.

Tests using indicator plants are easy to do, cheap, quick and sensitive, and may detect viruses that are not detected by the other methods. The sap of some plants, including sweetpotato,

contain substances that can block virus detection by some tests (including ELISA). When testing for sweetpotato viruses, it is best to use the sap of an indicator plant onto which the plant to be tested has been grafted.

In this chapter, we outline tests with *I. setosa*. Chapter 6 covers ELISA and gives a short introduction to PCR.

The easiest and most reliable method for detecting sweetpotato viruses is to use an indicator plant such as *I. setosa*. Shoots are taken from plants under test and grafted onto the indicator plant. If symptoms develop, the plant can be tested using the International Potato Center (CIP) ELISA kit, to find out which of 10 viruses may be present. Using the kit directly on sweetpotato is less likely to give positive results, because sweetpotato has chemicals in its cells that can destroy the viruses when the leaves are ground up as part of the test.

5.1 Transferring plants from tissue culture to pots

Deflasking plants from tissue culture (Figures 16 and 17) and transferring them to sterilised soil or potting mix must be done with care. The plants are delicate, having been grown at high humidity and at relatively high temperatures, and the leaves and roots are fragile. They are easily damaged unless they are allowed to adjust gradually to the new conditions.

It is important that the plants are placed in a screen house, glasshouse or screened compartment to keep them free from insects; they should also be kept away from plants that might be infected with viruses.

What equipment do I need?

- A bucket of clean water, or clean running water
- Potting mix
- A number of 10-cm diameter pots
- A spoon or similar implement to remove plants from the culture bottles
- Clear plastic sheeting or plastic bags
- Labels and pen
- Personal protective equipment (Appendix 3)

All the activities described below must be done in an insect-proof screen house, glasshouse or screened compartment.

The steps in transferring plants in tissue culture to pots are as follows:

- Working with one variety at a time, remove the plantlets from the bottles. Use a clean spoon, spatula or similar instrument to loosen the gel in the bottle, and gently tip the plantlets out. Be careful not to detach the roots.
- Gently rinse most of the gel from the roots in a bucket of clean water, or under slowly running water (Figure 17). It does not matter if some of the gel remains attached to the delicate roots.
- Do not trim the roots.
- Place the plants in 10-cm pots containing a commercial potting mix or sterilised soil mix, or plant them in punnet trays (Figure 18).
- Water in thoroughly, so that there are no air pockets around the roots.
- Label the pots accurately with collection number and/or name, and the date transferred to pots from tissue culture.

- Cover each pot with a clear plastic bag, or put the pots in an enclosure made of clear plastic sheeting to maintain high humidity (Figure 18); these should be inside a screen house, glasshouse or screened compartment.
- After the initial watering, there may be no need to water again for 5–7 days, depending on the temperature, but check every day. It is very important not to water the plants so often that the soil remains continuously wet; this is especially important in the first few weeks, when their roots are still developing. Waterlogged young plants are very susceptible to fungal infections, which can be lethal (see Section 1.6). When conditions are right, growth is rapid (Figure 18).

Figure 16
Plants in tissue culture, ready to be transferred to pots

Figure 17
A deflasked plant being rinsed in clean water to remove the tissue culture medium from around its roots; alternatively, wash the roots under slowly running water

a. Insect-proof screened bench with plastic sheeting to maintain high relative humidity inside a screen house

b. Tissue culture plants growing in punnet trays in a screened compartment on a bench

c. Plants in punnet trays 2 weeks after deflasking, and ready for transfer to larger pots before grafting onto *Ipomoea setosa*

d. A screened compartment with plants inside

Figure 18
Transferring tissue culture plants to potting mix

5.2 Virus indexing: indicator plants

Ipomoea setosa (Figure 19) is a wild relative of sweetpotato. It is used widely as an indicator plant because it is susceptible to sweetpotato viruses and shows symptoms clearly. This manual demonstrates grafting onto *I. setosa,* as well as serology using the CIP nitrocellulose membrane (NCM) ELISA kit.

What equipment do I need?

A 'grafting kit' (Figure 20), which includes the following:

- Scalpel handle (no. 3) and scalpel blades (no. 11), or sharp knife
- Paper towel
- Tape for grafting (plumbers' tape is ideal)
- Scissors, 150 mm
- Pencil and plastic plant tags to label pots
- Pen and data sheet to record grafted plants
- 30-cm bamboo stakes
- Laboratory-grade ethanol (70%) or isopropyl alcohol
- Spirit lamp or candle, and matches
- Household bleach (diluted to a concentration of 1.5%; see Appendix 1)

Other items of equipment:

- Plastic bags long enough to cover the whole plant and top of the pot
- 10-cm diameter plant pots or punnet trays
- Sulfuric acid for seed scarification
- Plant stakes, about 30 cm long
- Data sheets with columns to record collection name and/or number, date of graft and symptoms (Appendix 8)

5.2.1 Growing *I. setosa*

Ipomoea setosa can become a weed, so make sure it does not 'escape'. It is only for research purposes. All seeds and plants should be kept in the screen house, glasshouse or screened compartment, and the plants should be destroyed after use.

The steps for growing *I. setosa* are as follows:

- *I. setosa* plants should be grown and kept in a separate screen house, glasshouse or screened compartment, away from virus-infected and healthy sweetpotato plants.
- It is best to collect and use fresh seed, which usually gives good germination (98%) without scarification. If fresh seed is not available, scarify the seed by covering it with concentrated sulfuric acid (98%) for 25 minutes, and then rinse with water several times.
- Place one *I. setosa* seed in each 10-cm pot containing commercial potting mix or sterilised soil mix, and cover with about 1 cm of mix.
- Water thoroughly.
- After about 4–6 weeks, depending on the ambient temperature, the first true leaves will be fully expanded, and the *I. setosa* plants will be ready for grafting (Figure 19).
- Use five *I. setosa* seedlings for each sweetpotato plant to be tested. On each plant, make two grafts: one terminal or end cleft, and one side or veneer. There will be 10 grafts for each sweetpotato plant under test.
- Sow extra *I. setosa* seed to have plants as 'controls'— these plants are compared with those that are grafted with sweetpotato. There are two types of controls: negative (left ungrafted or grafted with healthy *I. setosa*) and positive (grafted with shoots from plants that are known to be virus infected; see Section 5.2.6).

Figure 19
Seedling of *I. setosa* ready for grafting (about 4 weeks old)

Figure 20
A kit for grafting shoots of sweetpotato to the indicator plant, *I. setosa*

5.2.2 Grafting: terminal (end cleft) graft

Thin plumbers' tape is best for binding the graft—it is soft and flexible, making it easier to get a good seal between the scion (sweetpotato shoot) and rootstock (*I. setosa*). It is also the cheapest! It can be purchased from hardware or plumbers' supply stores.

Terminal (end cleft) grafts are illustrated in Figures 21 and 22.

Procedure 4 (page 80) illustrates the steps for terminal grafting, which are as follows:

- Use sterile scissors or a sterile scalpel blade to cut horizontally through the stem of an *I. setosa* seedling just below the second true leaf.
- Use a sterile scalpel blade to cut the stem vertically into two equal parts about 15 mm deep.
- Remove a single node cutting from a sweetpotato to be tested, and trim the stem below the bud into a wedge about 15 mm long.
- Try to match the size of the wedge to the size of the cut made in the *I. setosa* seedling.

- Place the sweetpotato wedge in the cut *I. setosa* stem.
- Wrap plumbers' tape or grafting tape around the union between the sweetpotato and the *I. setosa* seedling. Bind the tape firmly, to reduce the chance of air pockets between the join of the scion (sweetpotato) and rootstock (*I. setosa*).

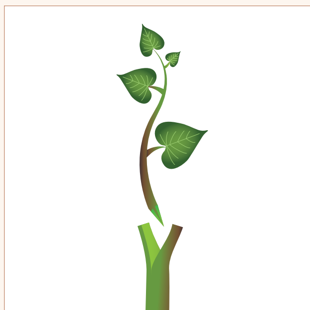


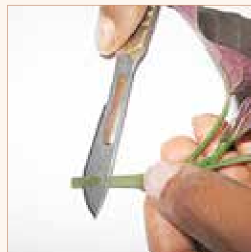
Figure 21
A terminal (end cleft) graft. The shoot at the top is sweetpotato, with the stem cut to a wedge to fit the split *I. setosa* stem.

Figure 22
A close-up of a terminal (end cleft) graft

Procedure 4 Stages in making a terminal (end cleft graft) between shoots of sweetpotato and the indicator plant *I. setosa*

a. Removing the terminal shoot on *I. setosa*

b. Cutting the stem into two, to a depth of 15 mm



c. Trimming the stem of a sweetpotato shoot to a wedge

d. Putting the sweetpotato shoot into the *I. setosa* stem

e. Binding the graft tightly with plumbers' tape

f. Covering the grafted plant with a plastic bag to keep the relative humidity high and prevent wilting while the scion and rootstock combine

5.2.3 Grafting: side (veneer) graft

Make sure that the shoot for the side (veneer) graft is taken from the same sweetpotato plant that provided the shoot for the terminal (end cleft) graft. Remember, there should be five plants for each sweetpotato variety under test.

Side (veneer) grafts are illustrated in Figures 23 and 24.

Procedure 5 (page 82) illustrates the steps for side grafting, which are as follows:

- On the same *I. setosa* seedling as the terminal (end cleft) graft, make a 15 mm diagonal cut in the stem below the cotyledons, so that the cut finishes at the centre of the stem.
- Remove a shoot from the same sweetpotato plant used for the terminal (end cleft) graft and trim the lower portion into a wedge.
- Place the shoot into the split stem of the *I. setosa* seedling.
- Wrap plumbers' tape or grafting tape around the join between the sweetpotato and *I. setosa* seedling. Bind the tape firmly, to reduce the chance of air pockets between the scion (sweetpotato) and rootstock (*I. setosa*).

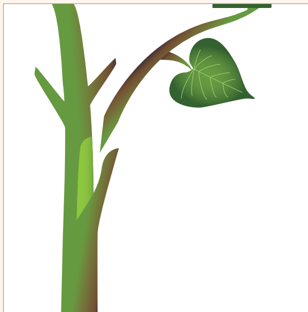


Figure 23
A side (veneer) graft. The lower part of the stem of the sweetpotato is cut as a wedge to fit into the diagonal cut made into the *I. setosa* stem.

Figure 24
A close-up of a side (veneer) graft

Procedure 5 Stages in making a side (veneer) graft between shoots of sweetpotato and the indicator plant *I. setosa*

a. Making a diagonal cut in an *I. setosa* stem to hold the sweetpotato shoot

b. A sweetpotato shoot taken from the same plant that provided the scion for the terminal (end cleft) graft

c. The shoot trimmed to a single node and the stem cut to a wedge

d. The sweetpotato shoot inserted into the *I. setosa* stem and being bound in place with plumbers' tape

5.2.4 Grafting control plants

In addition to growing plants for pathogen testing, control plants must also be grown. Control plants are plants that are either known to be free of virus (negative control) or plants that are known to have virus (positive control).

The negative control can be an *I. setosa* plant that is grafted with a cutting from another *I. setosa* plant that has not been exposed to virus or virus vectors (e.g. whitefly, aphids). This control will show that the *I. setosa* seeds are healthy, or will indicate any environmental effects that may be confused with virus symptoms on the test plants. Virus symptoms occurring on a negative control would indicate a breakdown in the isolation of the growth areas (screen house, glasshouse or screened compartment) from virus vectors. In this situation, all plants would have to be retested.

The positive control is an *I. setosa* plant grafted with shoots from a sweetpotato plant that is known to be virus infected. In other words, it is a plant that will show symptoms. If it does not show symptoms, then all other sweetpotato plants that failed to produce symptoms on *I. setosa* need to be retested.

5.2.5 After grafting

After grafting the *I. setosa* plants with the two types of grafts, support the plant with a bamboo stake or slim stick about 30 cm long, tied to the stem with plumbers' or grafting tape (Figures 25 and 26). Label it accurately with the:

- sweetpotato name and/or number
- date of grafting
- initials of the person doing the grafting.

Repeat these procedures on the remaining four plants to be tested.

Incubate the plants as follows:

- Place the pots in saucers or trays on a bench in the screen house, glasshouse or screened compartment.
- Keep the plants at high relative humidity for the first 4–7 days by covering them with plastic bags or keeping them inside a screened compartment lined with plastic sheets. Avoid putting them in direct sunlight. If it is hot, with temperatures

above 32 °C, cover the plants with shade cloth and/or place them on the floor below the benches.

- Remove the plastic bags or sheeting once plants have started to grow.
- Check daily to ensure that the plants have sufficient water, especially in hot weather, but do not overwater (see Section 1.6).
- Check that the grafted sweetpotato shoots remain alive (Figure 25). If they die in the first week after grafting, the plants cannot be recorded as negative (i.e. not having virus). If none of the other plants showed symptoms, the test must be repeated.
- Virus symptoms (if any are to occur) should appear within 14–21 days, but they can appear as early as 3 days and as late as 42 days, depending on the virus(es), daily temperatures and day length.

5.2.6 Key points about grafting

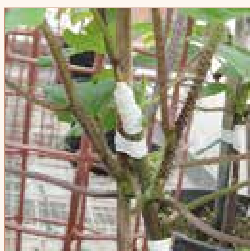
- *I. setosa* seeds do not contain viruses. However, it is important to use control plants to check that the seeds are healthy and not showing signs of environmental effects, which could be confused with virus symptoms on the test plants.
- *I. setosa* seedlings need to be kept in an insect-free environment, so that they do not become infected by viruses while testing is in progress.
- Every sweetpotato plant to be indexed needs to be grafted onto five *I. setosa* seedlings, with two grafts on each plant: terminal (end cleft) and side (veneer). This means that each sweetpotato plant tested provides 10 shoots for grafting.
- Each sweetpotato plant under test needs to be grafted onto *I. setosa* at least twice, preferably three times, about 1–2 months apart.
- After grafting, the plants need to be kept under humid, shaded conditions for 4–7 days to enable the union to establish between the sweetpotato and the *I. setosa*.

a. *Ipomoea setosa* plants 1 week after plastic bags were removed, and the grafted plants placed on benches with trellises to support the new shoots

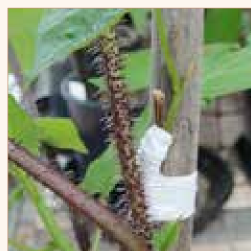
b. Grafted plants 6 weeks after grafting

c. A successful terminal (end cleft) graft

d. A successful side (veneer) graft



e. A plant with both types of graft successful



f. A plant in which part of the original graft has died, but a new shoot has emerged

g. A dead graft. The test has to be repeated

Figure 25
Successful and unsuccessful sweetpotato grafts on *I. setosa*

- In hot climates, the bags or sheeting may be lifted a little to allow some air flow around the plants, to stop them overheating while still maintaining high humidity levels around the plant.
- Remove the plastic bags or sheeting once plants have started to grow.
- The scalpel must be dipped in ethanol/alcohol and flamed between each graft.
- There must be a positive and a negative control (see Section 5.2.4).
- Only healthy *I. setosa* seedlings should be used for grafting. Do not use *I. setosa* plants showing chimeras or other potentially confusing symptoms.
- The grafted plants need to be labelled accurately.

Figure 26 summarises the grafting procedures.

5.2.7 Checking for symptoms

Symptom expression is usually very reliable with *I. setosa*. However, poor nutrition, high temperatures and plants with chimeras may cause confusion. It is important to always have negative and positive controls; these can be used for comparison if you are unsure.

- Symptoms on any of the five grafted *I. setosa* plants mean that the sweetpotato plant under test is infected, and heat therapy and meristem culture will need to be repeated.
- Virus symptoms come and go, and sometimes this happens quickly. Grafted plants therefore need to be checked at least twice weekly from about 1 week to 6 weeks after grafting.
- The types of symptoms that can be seen on *I. setosa* leaves grafted with sweetpotato shoots are shown in Figure 27.
- If the sweetpotato cuttings die in the first week, dispose of the plants and repeat the test, unless clear symptoms of infection appear before death occurs.

- After 7 weeks, dispose of the plants by bagging and freezing them, or by burning. Since the plants might be infected with virus, do not throw them onto rubbish piles near shade houses or glasshouses. The plants could become infested with insect vectors that could spread the viruses they contain. *I. setosa* has the potential to rapidly spread as a weed. Freezing or burning it will ensure that this does not happen.

a. *Ipomoea setosa* ready for grafting

b. Plant with both terminal and side grafts, supported by a bamboo stick

c. Plants being covered with plastic bags to create high humidity

d. Plants covered with plastic bags on a screened bench

Figure 26
Summary of grafting procedures, and method of incubation

If heat therapy needs to be repeated, it is best to use the plant that has already been heat treated. A heat-treated plant may have a lower amount of virus than one that has not been treated. Where there are mixtures of viruses, it is also possible that one or more has been eliminated by the heat therapy.

It is important to repeat the heat therapy as soon as possible, because the amount of virus in the plant will increase as growing conditions revert to normal. However, consider the condition of the plant, and wait until it has recovered from the first treatment before treating it again.

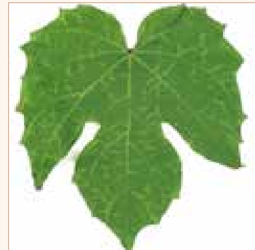
a. Healthy plant

b. SPFMV: mottling, commonly seen on lower leaves of *I. setosa*

c. SPFMV: vein clearing on midrib and other major veins of mid-section leaves

d. SPFMV: vein clearing and feathering along veins, and distortion

e. SPFMV: vein clearing and feathering along veins



f. SPFMV: vein clearing along main veins and veinlets, throughout the leaf

Figure 27

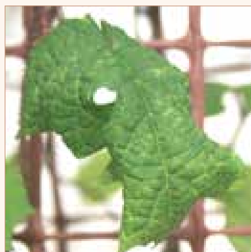
Symptoms (spots, vein clearing, cupping, necrosis) of sweetpotato virus infections on *I. setosa* from grafts carried out at Gatton Research Facility

continued next page

g. SPFMV: vein clearing, creating a net effect

h. SPFMV: fine vein clearing, giving the appearance of a net; sometimes the leaf appears silverish

i. SPCaLV: chlorotic spots



j. SPCaLV with begomovirus: small chlorotic areas along veinlets and leaf cupping

k. SPFMV and other viruses: severe leaf distortion and vein clearing

l. Begomovirus complex: leaf cupping and other distortions at the leaf margin

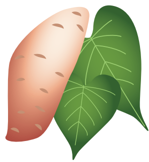
m. SPCaLV and begomovirus: necrosis due to multiple infection

n. Sweetpotato little leaf phytoplasma: fine vein clearing throughout the leaf

SPFMV = sweetpotato feathery mottle virus; SPCaLV = sweetpotato caulimo-like virus

Figure 27 (continued)
Symptoms (spots, vein clearing, cupping, necrosis) of sweetpotato virus infections on *I. setosa* from grafts carried out at Gatton Research Facility

Notes



Tests for viruses: ELISA and PCR

There are two reasons for grafting sweetpotato onto *Ipomoea setosa*:

- *I. setosa* is sensitive to many, perhaps all, viruses that infect sweetpotato. This means that even a new virus, for which there is no immunological (ELISA—enzyme-linked immunosorbent assay) or molecular (PCR—polymerase chain reaction) test, will produce symptoms. The symptoms are a warning that virus may be present, and the plant should be treated to remove the virus.
- ELISA tests on sweetpotato leaves are not completely reliable. Transferring the viruses into *I. setosa* via a graft solves this problem.

This chapter describes how the ELISA test is done, and gives a short introduction to the PCR test.

6.1 Virus indexing: ELISA

ELISA is a biochemical technique that is used to detect the presence of specific substances, such as antigens or antibodies, in blood or plant sap, for instance. The ELISA technique has several variations.

In this manual, we illustrate the steps in indirect ELISA (Procedure 6, page 93). This is the method used in the International Potato Center (CIP) kits, which are designed to detect 10 sweetpotato viruses. The detailed methodology is not given, as it is best to follow the instructions that come with the kits. Here we provide some tips based on our experience with using the kits.

We recommend that the CIP nitrocellulose membrane (NCM) ELISA kit is used by anyone who wishes to do routine testing for sweetpotato viruses.

6.1.1 Steps in the NCM-ELISA test

The procedures in NCM-ELISA are as follows:

- Blot *I. setosa* sap samples onto an NCM. The coat of the virus particles is made up of proteins, which bind to the surface of the membrane.
- Add a blocking agent, such as milk powder, to the membrane. The blocking agent fills the empty spaces on the membranes that are not taken up by virus particles contained in the *I. setosa* sap. This prevents false positive results.
- Add antibodies to the virus proteins on the membranes; these attach to the virus particles bound to the membranes. (If there were empty spaces on the membrane, the antibodies would attach to them, and could give a positive result even without the presence of virus.)
- Add antibodies to the virus antibodies. These are called conjugate antibodies and are produced in rabbits. They attach to the antibodies that are already attached to the virus particles. Importantly, they have an enzyme attached to them.

- Add a chemical (called the substrate) that reacts with the enzyme present on the conjugate antibodies and causes it to change colour. This colour change indicates a positive reaction, meaning that the virus is present.

Procedure 6 Steps in the NCM-ELISA procedure. Reproduced with kind permission of the International Potato Center (CIP), Peru (taken from the instruction manual for the CIP NCM-ELISA kit for sweetpotato virus detection).

Healthy
Infected

Step 1
Add the *I. setosa* sap
Plant sap

Step 2
Add the blocking agent (milk)
Blocking

Step 3
Add virus antibodies
Antibody 1 (virus-specific antibody)

Step 4
Add antibodies to the virus antibodies
Antibody 2 (antibody conjugate with enzyme)

Step 5
Add the substrate which reacts with the enzyme attached to the antibodies to the virus antibodies
Substrate

Negative
Positive

6.1.2 Performing the NCM-ELISA test

What equipment do I need that is not included in the kit?

- Plastic bags for collecting leaves and grinding samples
- Laboratory-grade ethanol (70%) or isopropyl alcohol
- Household bleach (diluted to a concentration of 1.5%; see Appendix 1)
- Bottles, beakers or plastic containers in the following sizes which will hold the following solutions:
  - 2 × 2 L bottle
    - Tris-buffered saline (TBS)
    - Tween–Tris-buffered saline (T-TBS)
  - 1 × 1 L bottle
    - antibody buffer
  - 4 × 500 mL bottle
    - extraction buffer
    - blocking solution
    - conjugate solution
    - substrate buffer
  - 10 × 100 mL beakers (each of the antibodies is mixed with antibody buffer; this is called the antibody solution. There is a different antibody solution in each beaker)
- Pipettes: 100 µL to 1,000 µL, and 10 µL to 100 µL, and disposable tips to fit
- Paper towels
- 10 small plastic bags, or flat-bottomed plastic containers (a membrane will be placed in each bag or container, together with 30 mL of reagents)

- A thick test tube or round piece of wood about 15 cm long—for example, a section of a broom handle—for macerating leaf samples
- Distilled water, at least 8.5 L
- Sterilised forceps and scissors
- Clips, pegs or similar, to hang the plastic bags containing the macerated leaf and sap
- Pencil and permanent marker pen
- Data recording sheets
- Personal protective equipment (Appendix 3)

Note: The names used in this list are those used in the instruction manual for the CIP NCM-ELISA kit.

Store all reagents in a sealed plastic bag at 4 °C in a refrigerator. They will be stable for 6–8 months.

If you use flat-bottomed containers (rather than plastic bags) to treat the membranes with reagents, make sure the base is **flat** and not indented, ridged or raised; otherwise, the membranes may not be treated evenly.

Wear a lab coat and surgical gloves at all times in the laboratory when handling leaf samples and membranes.

The instructions in the kit suggest that the test is done over 2–3 days, so give yourself the time needed; do not hurry!

6.1.3 Deciding on the number of *I. setosa* plants to test

Shoots from a single heat-treated, meristem-grown sweetpotato plant were grafted onto five *I. setosa* plants. Now you have to decide how many of these plants to test.

Ideally, you would test every plant, if time and resources were not limiting. If they are limiting, you might want to consider some options:

- If symptoms are not obvious on any of the five *I. setosa* plants, sample and blot leaves from three plants 2–3 weeks after grafting, and again 4–6 weeks after grafting.
- If symptoms are obvious on all five *I. setosa* plants, sample and blot two of them 2–3 weeks after grafting.
- If symptoms are obvious on one to four plants, sample and blot two plants 2–3 weeks after grafting, preferably those with symptoms, and sample and blot another two 4–6 weeks after grafting.

As antibody supplies in the ELISA kit are limited, leaf samples are often blotted then stored (for up to 6 months) until there is a sufficient quantity to run the complete test.

6.1.4 Sampling *I. setosa* leaves

The steps in sampling *I. setosa* leaves, illustrated in Procedure 7 (page 97), are as follows:

- Wearing gloves, remove three whole leaves from the *I. setosa* plant to be tested: one from the bottom, one from the midsection, and one from near the top. This is done because any virus present may not be evenly distributed throughout the plant.
- If there are leaves showing virus symptoms, choose these.
- Place the three leaves in a sealed plastic bag.
- Repeat the process on the remaining *I. setosa* plants grafted with shoots from the same sweetpotato variety that you have decided to test.
- Use a permanent marker pen to label the plastic bags. To ensure that your labels will not rub off during the leaf grinding process, label near the top of the bag. Leaves in bags should be taken to the laboratory as soon as possible and kept

Procedure 7 Sampling leaves of *I. setosa* for virus testing

a. Choosing the leaves to sample (note the use of gloves)

b. Removing a leaf

c. Labelling the plastic bag (at the top of the bag)

d. Sealing the labelled plastic bag

cool at all times. If it will take an hour or two to reach the laboratory, place the leaves in an esky or box with an ice brick or cold pack.

- When labelling, ensure that the label provides enough detail to fully identify the sample.
- Only collect as many samples as you can process in a day. Samples should be blotted to the membranes on the day they are collected.

- Change your gloves regularly when sampling leaves. Change them immediately if they become contaminated with sap. Inserting the leaf into the plastic sample bag before removing the gloves will minimise the risk of cross-contamination occurring.

6.1.5 Preparing the extraction buffer

The steps in making up the extraction buffer, illustrated in Procedure 8 (page 99), are as follows:

- Rinse all the bottles, beakers, dishes, and so on, in distilled water before use. They should have been previously soaked in household bleach for a few hours or overnight, triple rinsed with tap water, rinsed with distilled water and allowed to dry.
- Make up the extraction buffer following the directions in the CIP NCM-ELISA kit. The extraction buffer has to be made up fresh each day, as it degrades after 12 hours. (In contrast, TBS and T-TBS solutions can be kept in the refrigerator for 3–4 months.)
- Make up smaller amounts of extraction buffer for smaller numbers of samples—for example, for about 70 samples, make up 250 mL of extraction buffer by adding 0.5 mg of sodium sulfite to 250 mL of TBS.

6.1.6 Grinding *I. setosa* leaves

The steps in grinding the leaves, illustrated in Procedure 8 (page 99), are as follows:

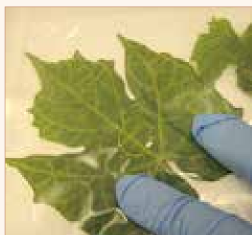
- Using the open end of a test tube, cut a disc from each of the sampled *I. setosa* leaves through the plastic bag. Aim to take the disc from where the main veins converge above the petiole. Take care not to cut through or break the plastic bag!
- Open the plastic bags and remove the unwanted leaf tissue using forceps sterilised in laboratory-grade ethanol (70%) or isopropyl alcohol. Try not to open the plastic bag more than necessary. If samples were collected in a large plastic

Procedure 8 Making up extraction buffer and grinding the leaves from *I. setosa*. Note that gloves are used throughout these steps.

a. Adding HCl to adjust the pH of the extraction buffer (TBS plus sodium sulfite)

b. Sampling an *I. setosa* leaf

c. Cutting out a circular leaf disc using a test tube pressed against the outside of the plastic bag



d. The cut leaf disc. Note its position: all samples are cut from where the main veins converge above the petiole

e. Measuring extraction buffer by pipette

f. Adding extraction buffer to the plastic bag containing the leaf disc

g. Rolling the test tube over the leaf discs to break the tissue and release the cell contents

h. Grinding the leaf discs

i. Partially ground leaf discs

j. Plastic bag hung to allow the ground leaf discs to settle

bag, the leaf discs can be transferred carefully into a smaller plastic bag for grinding; it is very important to label the new bag correctly.

- Dispose of the unwanted leaf tissue by freezing it to destroy leaf cells and virus, and then place it in the rubbish bin or burn it.
- Manipulate the discs so that they are at the bottom of the bag, then add extraction buffer at the rate of 1 mL per leaf disc (3 mL in this case).
- Roll a thick-walled test tube or a piece of round wood over the plastic bag to grind the leaf discs, taking care not to puncture the bag.
- Hang the plastic bags containing the ground tissue on a line with pegs or paper clips, at an angle, so that sap falls to one corner at the bottom of the bag.

Do not grind too many samples at one time; the ground samples must be blotted within 30 minutes. Do a maximum of 20 samples and then blot them; once dry, the membranes can be stored in the refrigerator. Change your gloves after grinding and before blotting.

6.1.7 Preparing and blotting the membranes

The procedures for preparing and blotting membranes and other important steps of NCM-ELISA, which are illustrated in Procedure 9 (pages 102–103), are as follows:

- Wear a new set of gloves after grinding the leaf discs and before handling the membranes.
- Lay out the 10 membranes on top of paper towels. The membranes are ruled into squares and have a right side and a wrong side; this will be evident if you look carefully at the membranes under light. Make sure that the side facing up is the one that shows the more pronounced lines.
- Using a sharp pencil (pencil marks will not wash off during the test and will not damage the membranes), label each of the

10 membranes with the date, and the name of the virus for which it will test (one membrane tests for only one virus).

- Place other paper towels on top of the membranes to protect them from possible contamination. Remove the top paper towels when you are ready to blot.
- Prepare a data sheet to record samples as they are blotted to the membranes (Table 1a). The sap from a bag can be blotted on any square on the first membrane, but once that has been decided, blots on the other membranes must be in the same position.
- Since proteins are trapped by the membrane surface, it is important not to contaminate it. Avoid breathing on the membranes or touching them with ungloved hands. If you have long hair, tie it back.
- Record the collection number (or name) of the sweetpotato variety and the plant number of the *I. setosa* plant on the data sheet. This will indicate which square on each of the membranes will be blotted.
- Cut off the bottom corner of the bag—opposite the side where the sap solution has collected—or cut the top of the bag diagonally opposite the sap solution. Using the bag as a cup, pipette out 15 µL of clear sap solution, and place it in one square of the membrane. Repeat this for each of the 10 membranes. The square blotted on each membrane must correspond with the name of the square marked on the data sheet.
- Take your time when blotting, and do not rush, as mistakes can easily occur. If mistakes occur, they must be recorded as soon as possible. For example, if a blot is accidentally placed on the wrong square or runs over into another square, write an X in pencil over the square and on the corresponding squares of each of the 10 membranes and record it on the data sheet (Table 1b).
- Discard the remaining sap in the bag once the 10 membranes have been blotted (or 20 membranes, if you are doing a backup set of membranes).

Procedure 9 Running the ELISA test—some important steps

a. Blotting the membranes with sap

b. A partially blotted membrane

c. Adding blocking agent to the membranes

d. Removing the blocking agent

e. Preparing to add the antibodies to the 10 viruses to the membranes, in separate containers

f. Shaking the membranes with antibodies

g. Removing the antibodies

h. Drying the membranes between paper towels

i. Making up the conjugate buffer plus antibody (to the virus antibodies)

continued next page

Procedure 9 Running the ELISA test—some important steps (continued)

j. Adding the conjugate antibody preparation to one membrane

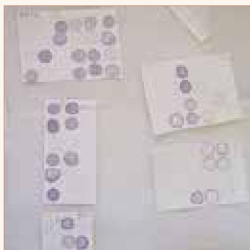
k. Adding conjugate antibody preparation to all 10 membranes

l. Membranes in conjugate antibody preparation

m. Discarding the conjugate antibody preparation

n. Membranes in the substrate buffer (note that they have been placed together for this step)

o. Washing the membranes under the tap



p. Purple dots showing presence of virus

- Replace the pipette tip with a new tip for each sample, and repeat the procedures listed above until all the samples have been blotted onto the membranes.
- After blotting, allow the membranes to dry. Then place the rectangular filter papers (in the kit) between them, and place them in a sealed plastic bag. To avoid moulds caused by moisture, place ½–1 teaspoon of silica gel in the bag. The bag can be kept at room temperature or in a refrigerator until you have sufficient samples to carry out an ELISA test. Aim to conduct the ELISA test no later than 6–12 months after blotting.

It is a good idea to blot an extra set of membranes as a backup. If the first test fails, you can rerun it with the spare set of membranes. Alternatively, you could send the spare set to another laboratory for testing.

It is very important to make a data sheet (see Table 1a–c) and then to follow the plan on the data sheet. Remember that blots of sap solution from the same sweetpotato variety/*I. setosa* plant combination must be in the same position on each of the 10 membranes.

It is also a good idea to do multiple blots of each sweetpotato/*I. setosa* combination on each membrane, as this will increase the confidence of positive or negative returns (see some entries in Table 1c).

Table 1a Blank data sheet for recording positions of leaf samples as they are blotted onto the membranes

Table 1b Data sheet showing positions where sweetpotato leaf samples have been blotted onto the membranes

Honey Gold 170 plnt A	L131 333 plnt A	Purp Cream 329 plnt A	Honey Gold 170 plnt A	Allys W 313 plnt A	Allys W 313 plnt A	L18 318 plnt B	@Hernandez 164 plnt A	Wanmun 311 plnt B	L49 303 plnt B	Eclipse 243 plnt A	Snowhite 101 plnt B
L18 321 plnt A	Ben kokie 315 plnt A	11 Rhrs 316 plnt A	Markham 307 plnt A	# Allys Red 85 plnt A	Honey Gold 170 plnt B	@ Darby 42 plnt B	Wanmun 311 plnt B	L131 333 plnt B	L49 303 plnt A	Pale Beau 301 plnt A	Collyam-bally 37 plnt A
Ben kokie 315 plnt A	L49 303 plnt A	L18 321 plnt A	Eclipse 243 plnt A	#Snowhite 78 plnt A	#Berry Custard 319 Plant A	#Berry Custard 319 Plant A	@Hernandez 164 plnt A	Moulin R 331 plnt B	L131 333 plnt B	Smiths Red 148 plnt B	Snowhite 101 plnt B
11 Rhrs 316 plnt A	x	@ Darby 42 plnt B	Pale Beau 301 plnt A	NG 7570 308 plnt A	Snowhite 101 plnt A	Pale Beau 301 plnt B	#Snowhite 78 plnt A	@Hernandez 164 plnt B	Purp Cream 329 plnt B	Pale Beau 301 plnt B	Collyam-bally 37 plnt A
Mag Gold 326 plnt A	Moulin R 331 plnt A	Moulin R 331 plnt A	# Beni Aka 69 plnt A	11 Rhrs 316 plnt A	Markham 307 plnt B	Mag Gold 326 plnt B	Smiths Red 148 plnt A	11 Rhrs 316 plnt B	Mag Gold 326 plnt B	L3 89 plnt B	@ Darby 42 plnt A
Wanmun 311 plnt A	Smith's Red 327 plnt A	Purp Cream 329 plnt A	Markham 307 plnt A	# Beni Aka 69 plnt A	Honey Gold 170 plnt B	Smith's Red 327 plnt B	Smiths Red 148 plnt A	#Yellow R 325 plnt A	Purp Cream 329 plnt B	L3 89 plnt B	x
Mag Gold 326 plnt A	Smiths Red 327 plnt A	L3 89 plnt A	Ben kokie 315 plnt B	Ben kokie 315 plnt B	# Allys Red 85 plnt A	Markham 307 plnt A	L18 321 plnt B	@Hernandez 164 plnt B	Eclipse 243 plnt B	Eclipse 243 plnt B	@ Darby 42 plnt A
Wanmun 311 plnt A	L131 333 plnt B	Moulin R 331 plnt B	NG 7570 308 plnt A	# Yellow R 325 plnt A	L49 303 plnt B	Smiths Red 327 plnt B	L3 89 plnt A	Snowhite 101 plnt A	Collyam-bally 37 plnt B	Collyam-bally 37 plnt B	Smiths Red 148 plnt B

= positive controls; @ = negative controls; x = a mistake when blotting

Table 1c ELISA results for sweetpotato varieties tested in Table 1b

Honey Gold 170 plnt A	L131 333 plnt A	Purp Cream 329 plnt A	Honey Gold 170 plnt A	Allys W 313 plnt A	Allys W 313 plnt A	L18 318 plnt B	@Hernandez 164 plnt A	Wanmun 311 plnt B	L49 303 plnt B	Eclipse 243 plnt A	Snowhite 101 plnt B
L18 321 plnt A	Ben kokie 315 plnt A	11 Rhrs 316 plnt A	Markham 307 plnt A	# Allys Red 85 plnt A	Honey Gold 170 plnt B	@ Darby 42 plnt B	Wanmun 311 plnt B	L131 333 plnt B	L49 303 plnt A	Pale Beau 301 plnt A	Collyam-bally 37 plnt A
Ben kokie 315 plnt A	L49 303 plnt A	L18 321 plnt A	Eclipse 243 plnt A	#Snowhite 78 plnt A	#Berry Custard 319 Plant A	#Berry Custard 319 Plant A	@Hernandez 164 plnt A	Moulin R 331 plnt B	L131 333 plnt B	Smiths Red 148 plnt B	Snowhite 101 plnt B
11 Rhrs 316 plnt A	X	@ Darby 42 plnt B	Pale Beau 301 plnt A	NG 7570 308 plnt A	Snowhite 101 plnt A	Pale Beau 301 plnt B	#Snowhite 78 plnt A	@Hernandez 164 plnt B	Purp Cream 329 plnt B	Pale Beau 301 plnt B	Collyam-bally 37 plnt A
Mag Gold 326 plnt A	Moulin R 331 plnt A	Moulin R 331 plnt A	# Beni Aka 69 plnt A	11 Rhrs 316 plnt A	Markham 307 plnt B	Mag Gold 326 plnt B	Smiths Red 148 plnt A	11 Rhrs 316 plnt B	Mag Gold 326 plnt B	L3 89 plnt B	@ Darby 42 plnt A
Wanmun 311 plnt A	Smith's Red 327 plnt A	Purp Cream 329 plnt A	Markham 307 plnt A	# Beni Aka 69 plnt A	Honey Gold 170 plnt B	Smith's Red 327 plnt B	Smiths Red 148 plnt A	#Yellow R 325 plnt A	Purp Cream 329 plnt B	L3 89 plnt B	X
Mag Gold 326 plnt A	Smiths Red 327 plnt A	L3 89 plnt A	Ben kokie 315 plnt B	Ben kokie 315 plnt B	# Allys Red 85 plnt A	Markham 307 plnt A	L18 321 plnt B	@Hernandez 164 plnt B	Eclipse 243 plnt B	Eclipse 243 plnt B	@ Darby 42 plnt A
Wanmun 311 plnt A	L131 333 plnt B	Moulin R 331 plnt B	NG 7570 308 plnt A	# Yellow R 325 plnt A	L49 303 plnt B	Smiths Red 327 plnt B	L3 89 plnt A	Snowhite 101 plnt A	Collyam-bally 37 plnt B	Collyam-bally 37 plnt B	Smiths Red 148 plnt B

= positive controls; @ = negative controls; x = a mistake when blotting

6.1.8 Important points about NCM-ELISA

Some of the important points are emphasised below:

- Blot the membranes as soon as possible after grinding the samples, ideally within 30 minutes.
- Each membrane is sufficient for 96 blots.
- You do not have to use the entire membrane each time. You can cut the membranes into pieces. Just make sure you have enough squares for the number of blots that you need to make. Cut membranes with scissors, and store the remaining sheets.
- Change gloves regularly, including after the *I. setosa* plants belonging to each sweetpotato have been blotted.
- Use a new pipette tip for the sap of each set of *I. setosa* leaf discs.
- Multiple sets of 10 membranes (or cut pieces of membranes) can be analysed in the same NCM-ELISA test.
- Do not forget the controls: check that you have included them before adding the blocking solution.
- It is very important to label each of the 10 membranes with the name of each of the 10 viruses.
- Remember to record the date, collection numbers (or names) on a blotting data sheet (Table 1).

A sweetpotato indexing record sheet is presented in Appendix 8. This allows you to summarise the data from Table 1, adding the symptoms that developed on both the 'mother' plants and the *I. setosa* plants.

Allow plenty of time to make up the solutions and reagents required for the NCM-ELISA test. Also allow time for the rinses required between steps of the test. Making the solutions and rinsing could take up to two hours.

6.1.9 Important points about antibodies

- Antibodies must be kept cool at all times; the antibody buffer solution should be prepared immediately before use.
- Any leftover antibody must be returned immediately to the refrigerator.
- The total NCM-ELISA test takes a day and a half to complete (Table 2) (less if membranes have been blotted previously). As part of this, you may need to allow up to two hours for making solutions/reagents and doing the rinses required.

Table 2 An abbreviated schedule for carrying out NCM-ELISA

Time	Task
Day 1, morning	Sample the leaves and blot onto the nitrocellulose membranes
Day 1, afternoon	Place the membranes in blocking solution on a shaker for 1 hour; rinse once with TBS; add the antibody solution specific to each virus (in separate containers); shake for 3 hours (or leave on the shaker overnight)
Day 2, morning	Discard the antibody solutions; rinse membranes four times with T-TBS; dry them on paper towels
	Return membranes to their respective containers, and add conjugate solution to each one; place the containers on a shaker for 1 hour
	Discard the conjugate solution; rinse membranes four times with T-TBS; dry them on paper towels
	Return membranes to their respective containers and add colour development solution (made with the substrate buffer); place the containers on a shaker for 30 minutes
	Discard all solutions (except for the SPCSV membrane, which must remain on the shaker for another 30 minutes); rinse membranes in tap water; dry them on paper towels
	Interpret results

SPCSV = sweetpotato chlorotic stunt virus; TBS = Tris-buffered saline; T-TBS = Tween–Tris-buffered saline

Note: Membranes that have been blotted and tested by NCM-ELISA can be laminated and stored for a long time, probably for several years.

6.1.10 Cleaning up and preparing for the next test

Once the ELISA test is complete, treat all bottles, beakers, dishes and other equipment as follows:

- Wash in soapy water.
- Soak in household bleach for a few hours or overnight.
- Rinse in tap water at least three times.
- Rinse with distilled water.
- Leave to dry.

Before performing the next ELISA test, it is important to rinse all equipment again with distilled water.

6.2 Virus indexing: PCR

This manual does not describe in detail molecular methods of indexing plant viruses based on PCR. However, it does introduce the use of FTA cards for sending samples to laboratories where PCR is routinely used to identify plant viruses. FTA is a system that fixes and stores nucleic acids (DNA and RNA) directly from fresh tissues pressed into treated paper cards. The cards are then sent to a laboratory to be tested for viruses. The technique is explained in Chapter 7.

PCR is a technique that increases the amount of genetic material in a sample that initially contains very small amounts, so that it can be detected. The products of PCR are then separated by putting them on a gel and passing an electric current through it. Pieces of the genetic material settle at different positions, depending on their size.

The PCR test for a plant virus is a set of repeated steps consisting of:

- heating the target genetic material of the virus to split the two strands of nucleic acid

- binding small pieces of matching sequence, called primers to the target sequence
- making more of (i.e. amplifying) the target genetic material of the virus.

A diagram of these steps is provided in Figure 28. Many videos on the internet also illustrate the PCR technique. One that is relatively simple can be viewed at <www.youtube.com/watch?v=2KoLnIwoZKU>, but there are many others.

The power of PCR comes from the fact that both the original virus and the products of each PCR cycle can act as targets for the next cycle, leading to an exponential increase in the amount of viral genetic material. This feature, as well as the commercialisation of the process, has made PCR a popular method for virus detection.

The advantages of using PCR for detecting sweetpotato viruses are:

- it is very sensitive, allowing us to detect very small amounts of virus in a plant
- it is very specific, allowing us to identify exactly which virus group or individual virus is present in the plant
- it can be used to rapidly test the large number of plants required for clean planting material schemes
- it can be used to investigate new viruses that may be present in sweetpotatoes.

The virology group at the Queensland Department Agriculture, Fisheries and Forestry has been optimising PCR tests for a number of sweetpotato viruses. Those that can be detected routinely are sweetpotato feathery mottle virus, sweetpotato mild mosaic virus, sweetpotato virus G, sweetpotato leaf curl virus and cucumber mosaic virus. The group has degenerate PCR systems to detect members of the potyvirus and begomovirus groups. Other viruses of interest are sweetpotato latent virus, sweetpotato virus 2, sweetpotato chlorotic fleck virus and sweetpotato chlorotic stunt virus.

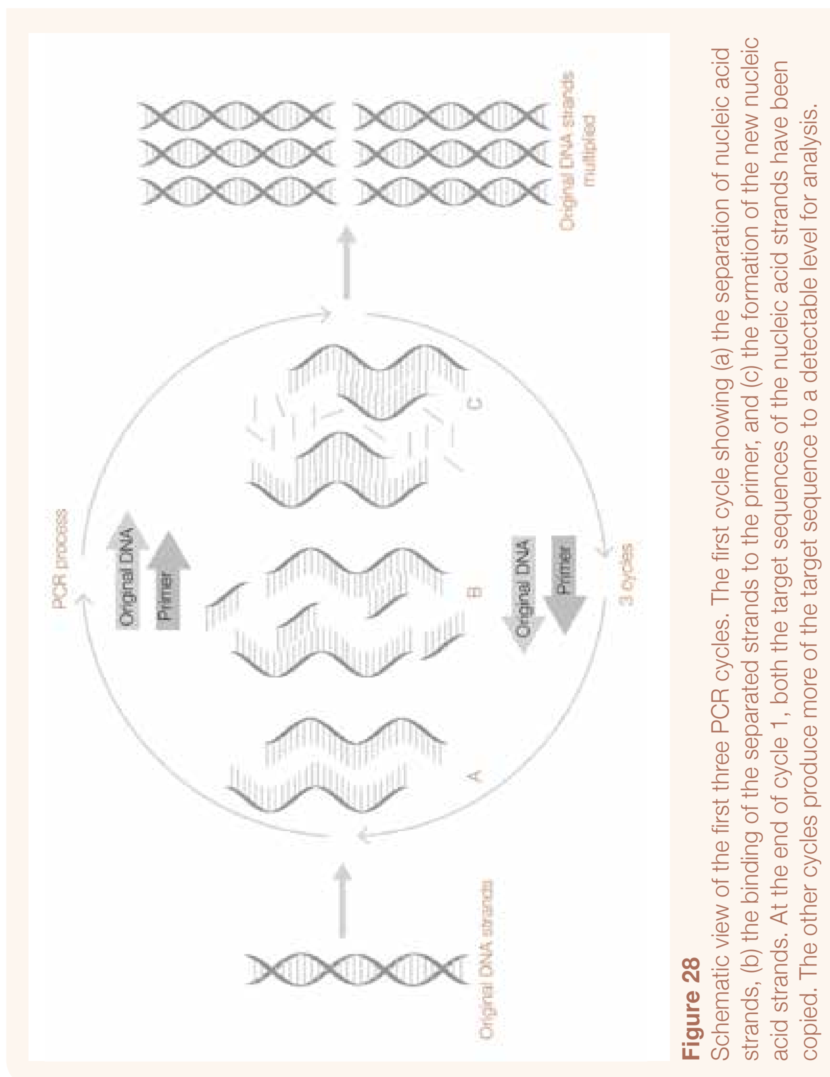
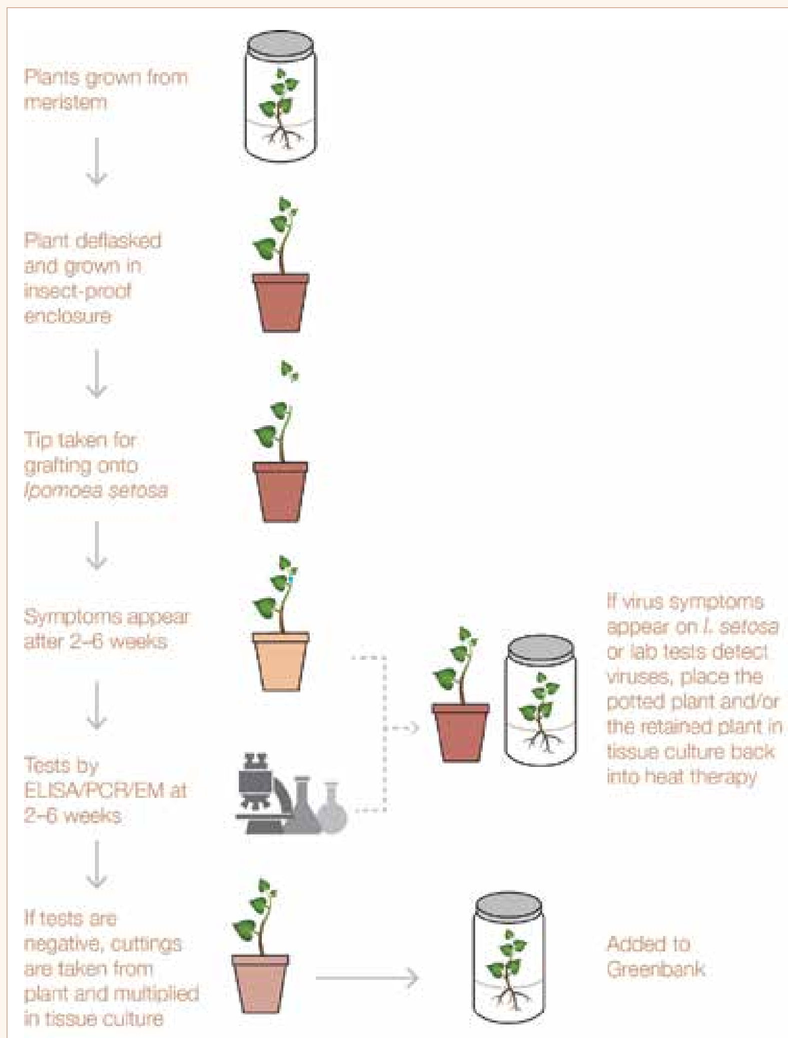


Figure 28
Schematic view of the first three PCR cycles. The first cycle showing (a) the separation of nucleic acid strands, (b) the binding of the separated strands to the primer, and (c) the formation of the new nucleic acid strands. At the end of cycle 1, both the target sequences of the nucleic acid strands have been copied. The other cycles produce more of the target sequence to a detectable level for analysis.

6.3 Putting it all together

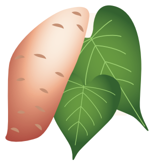
Figure 29 shows the scheme for applying heat therapy to plants to inactivate virus infections (Chapter 3), regrowing the plants from meristems (Chapter 4) and testing the regrown plants for viruses (Chapters 5 and 6).



ELISA = enzyme-linked immunosorbent assay; EM = electron microscopy; PCR = polymerase chain reaction

Figure 29
Virus indexing, showing the steps from the regrowth of a meristem taken from a heat-treated plant, through indexing on *I. setosa* and by ELISA, PCR or EM to conservation in a gene bank. If the indexing on *I. setosa* or by ELISA, PCR or EM shows that the plant is still virus infected, the steps have to be repeated.

Notes



Preservation: leaf samples, viruses and tissue cultures

We now come to the storage or preservation of leaves infected with viruses, and plants in tissue culture.

The previous chapter assumed that you would take fresh leaves from *Ipomoea setosa* indicator plants and immediately test them for viruses using the International Potato Center (CIP) nitrocellulose membrane (NCM)-ELISA (enzyme-linked immunosorbent assay) kit. This is the normal way that ELISA is carried out—on fresh leaves (although leaves can be stored in a refrigerator overnight).

However, you may want to delay the testing, perhaps because:

- you are doing a survey far from your laboratory, perhaps overseas, and you are concerned that the leaves will decay by the time you return
- you want to test for viruses that are not in the CIP NCM-ELISA kit (e.g. begomoviruses) and have to send the leaf samples to a different laboratory
- you know that ELISA is not an option, and that PCR (polymerase chain reaction) is the method to use. (Note that PCR, unlike ELISA, can be carried out on sweetpotato leaves, as well as on *I. setosa*.)

So how can you preserve the samples? There are two methods:

- Leaves can be stored using drying agents, silica gel or calcium chlorite.
- FTA cards can be used. These provide a safe, secure and reliable method for collecting, transporting and storing viral nucleic acid. The cards can be stored at room temperature for many years.

Apart from preserving leaves infected by viruses, you may want to preserve entire plants. One way is to make a field gene bank, but this is expensive, and the plants can be lost as a result of weather events, pests or diseases.

A better way is to conserve collections as sterile plants growing in tissue culture. This chapter provides some comments on doing this.

7.1 Preservation of leaf samples

7.1.1 Cutting and drying leaves

Leaves are preserved so that they can be tested by PCR at a later date, or so that they can be sent overseas for testing. DNA viruses, such as begomoviruses, are very stable and can be

successfully tested for in dried leaves after many years. However, RNA viruses, such as those in the potyvirus group, break down and may not always give a positive result from dried tissue.

What equipment do I need?

- New, self-sealing plastic bags
- Plastic tray that can be swabbed with ethanol/alcohol
- Paper towels
- Sharp scissors or scalpels and forceps (preferably, three sets of each—one set being used, one set sterilising and one set sterile and ready for use)
- Laboratory-grade ethanol (70%) or isopropyl alcohol
- Spirit lamp or candle, and matches
- Muslin or quarantine-grade netting to make small bags
- Staple machine and staples
- Silica gel
- Specimen bottles or small collecting vials (30 mL)
- Personal protective equipment (Appendix 3)

The steps in cutting up and drying the *I. setosa* leaves, illustrated in Procedure 10 (page 119), are as follows:

- Wearing gloves, collect leaf samples from plants to be tested. Place the leaves in a clean, labelled plastic bag.
- If you know that the plants you are testing are infected by virus, take leaves that are showing obvious symptoms; otherwise, sample leaves at the bottom, middle and top of each plant.
- Keep the samples cool while transporting them to the laboratory.
- In the laboratory, wear gloves, and place the leaves on a tray covered with a paper towel.

- Cut the leaves into small pieces with sterile scissors or a sterile scalpel blade (scissors are generally much quicker).
- Place pieces of leaf tissue into small bags made from muslin or quarantine net and secured with staples.
- Add a piece of paper with the plant name and number.
- Close the bags with staples.
- Place up to 4 bags in a new, large plastic bag.
- Add approximately 8 tablespoons of silica gel to the plastic bag.
- Seal the bag.
- Discard the paper towel, and disinfect the tray, scissors, scalpel and forceps with alcohol.
- Wipe gloves with ethanol/alcohol after cutting the leaves from each plant, and change gloves every 10–15 plants.
- Place a new paper towel on the tray.
- Soak the scissors or scalpel and forceps that have just been used in ethanol/alcohol.
- Flame the scissors or scalpel and forceps (the second set, which has been soaking in ethanol/alcohol) and allow to cool.
- Use the sterile, cool scissors or scalpel and forceps to repeat these procedures for a second lot of leaves to be preserved.

7.1.2 Drying the samples over silica gel

Do not put large amounts of leaf tissue in the bags. Follow the ratio of silica gel to leaf (8 tablespoons of silica gel per plastic bag, each plastic bag having 4 net bags each containing 3 leaves), or the leaf pieces will not dry quickly and will go mouldy.

The steps in drying samples of *I. setosa* leaves over silica gel are as follows:

- Change the silica gel daily, until the colour does not change (indicating that the leaf pieces have dried).

Procedure 10 Cutting and drying leaves

a. Cutting the leaves with sterilised scissors

b. Cuttings before they are placed in net bags—the pieces are about 1 mm wide and not more than 10 mm long

c. Placing the leaf tissue in a net bag

d. A net bag with leaves and a label before it is closed at the top by staples

e. Storing the net bags in a plastic bag, with silica gel

f. After drying, samples that have been transferred to small labelled bottles—these can be stored in a freezer

g. Different colours of silica gel before and after drying: purple and orange when dry, and pink and green when moist

- After leaves have dried, place a little silica gel in the bottom of a bottle, cover it with a piece of paper towel, and then add the leaf tissue from the net bags using sterile forceps.
- Use glass or plastic bottles with airtight lids.
- Label the bottle with the name of the variety, its number, the date the leaf was collected from the plant and where the variety was collected.
- The bottles can be placed in a freezer for long-term storage.
- Silica gel can be reused by drying it. Dry by placing it on a tray in an oven at 110 °C for 10–20 minutes, or by placing it in a heatproof glass beaker, to a depth of about 2–3 cm, and microwaving for 2–3 minutes.
- If using a microwave to dry the silica gel, check the gel at 1 minute intervals, and stir it before continuing with the drying process. As soon as the gel has returned to its original colour (see page 119, Procedure 10g), turn off the microwave and allow the gel to cool for 5 minutes. Be careful not to let it become too hot or it may fuse to the glass container.
- Silica gel is dried when it is back to its original colour, either purple or orange. Note that purple silica gel turns pink when moist; orange silica gel turns green.
- Since heated silica gel becomes extremely hot, **be careful**—leave beakers and oven trays in the microwave or oven to cool before handling.
- Once cooled, silica gel can be put in its original container (or any glass or plastic airtight container).
- Do not use plastic containers to heat silica gel—not even containers that are microwave safe. The silica gel becomes so hot that it will destroy microwave oven cookware; it will melt plastic containers and crack those made from glass. Only use laboratory borosilicate glass beakers.
- Oven trays are ideal if drying silica gel in an oven. They must be completely clean and free from all grease and oil, or the silica gel will stick to them.

An easy technique to remove small pieces of leaf tissue from the used silica gel is to place the silica gel in a sieve and, using a hairdryer on a low setting and placed below the sieve, blow the pieces of leaf out; otherwise, you can simply blow them out with your breath.

7.2 FTA cards

Samples containing DNA and RNA viruses can be placed on FTA cards and then sent for PCR testing. However, CIP has found that PCR tests on the nucleic acid of RNA viruses are only satisfactory for 1–2 weeks after samples are added to the cards. In contrast, DNA viruses such as sweetpotato begomovirus (family *Geminiviridae*) and sweetpotato caulimo-like virus (family *Caulimoviridae*) are not affected in the same way, and the method is suitable for sending them for analysis and/or storage.

What equipment do I need?

- FTA cards
- New, self-sealing plastic bags
- Clean bottles, soaked in household bleach (diluted to a concentration of 1.5%; see Appendix 1) for 20 minutes and rinsed in distilled water
- Parafilm—a plastic paraffin film with a paper backing
- Desiccant: small bags filled with silica gel
- Plastic bag sealer (if no self-sealing plastic bags are available)
- Permanent marker pens
- Personal protective equipment (Appendix 3)

Always wear gloves when handling FTA cards. Do not damage the surface of the FTA card when transferring samples.

Note that the FTA card must reach the laboratory for testing within 2 weeks to successfully detect RNA viruses; DNA viruses are much more resistant, and the card can be stored for testing at a later date.

7.2.1 Transferring samples to FTA cards

Wear surgical gloves and carry out the following steps (Procedure 11, page 123):

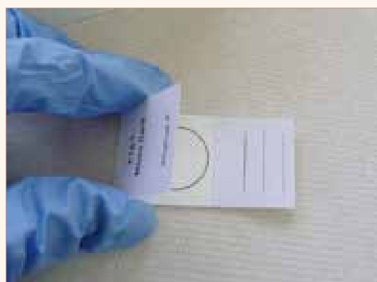
- Label the FTA card with the sample number in the space provided.
- Open the FTA card cover to expose the sample circle on the card.
- Choose a leaf with clear virus symptoms. Tear it so that it will fit inside a circle on the FTA card, and place it on the card, with the underside of the leaf facing downwards.
- If the FTA card does not have a cover sheet, place the parafilm over the leaf discs, but do not remove the printed paper overlay from the parafilm.
- Use a test tube, large marker pen or porcelain pestle to break the leaf discs, transferring the contents to the FTA card. Do this with moderate pressure, rubbing back and forth across the cover sheet or parafilm (with the paper overlay), and rubbing across the entire area of the leaf disc. The intention is to burst the cell walls of the leaf discs, transferring the contents to the card without damaging it.
- Verify that sufficient plant material has been transferred to the card by checking the back of the card: plant sap should be visible on the opposite side of the card—that is, there should be a green disc the size of the plant sample.

- Ensure that no large pieces of plant tissue remain on the FTA card. Peel off any large pieces with sterilised forceps, but do not scrape the card, as this may damage it.
- Immediately after transferring the sample to the card, allow the card to dry at room temperature for 1–2 hours. **Do not** heat the card to speed drying.
- Close the FTA card folder. Place it in a plastic bag, along with silica gel, and seal the bag.

Procedure 11 Using FTA cards for sending leaf nucleic acid samples

a. The equipment needed

b. Packs of cards (100) and a single card



c. The cover of a card and the target area for the leaf sample

d. Equipment needed for transferring leaf to the card

continued on next page

Procedure 11 Using FTA cards for sending leaf nucleic acid samples

e. A piece of leaf torn to fit the card, and covered with a clean piece of paraffin film or plastic wrap to prevent contamination

f. Transferring the leaf contents to the card by gently rubbing a test tube on the surface of the paraffin film

g. Discarded leaf (left); a labelled card with sap transferred (right)

h. Equipment for storing and/or sending the cards to another laboratory for testing

i. Sealing the plastic bag containing the card and silica gel

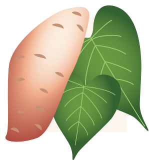
7.3 Long-term storage of sweetpotato plants in tissue culture

Generally, sweetpotatoes grow rapidly in tissue culture at about 25 °C. For large collections, with several duplicates per accession, subculturing accounts for a large part of the costs of conservation.

The length of time between subculturing can be extended in a number of ways:

- Place the sweetpotato cuttings, shoot tips or nodes in a tissue culture medium (usually Murashige and Skoog basal salt mixture, often abbreviated to MS) with reduced sugar levels, which will retard growth. The level of sugar reduction to use is usually learnt by trial and error, as sweetpotato varieties often behave differently. Useful starting points are to use 25% and 50% of the standard sugar requirement.
- Add mannitol to the medium (2%); this is an osmotic stress agent that slows the growth of the plants. This method is not recommended because there is evidence that plants become stunted permanently, or develop into callus, so that they cannot be rejuvenated.
- Reduce light hours to 8 hours daily.
- Incubate at cooler temperatures; some plants can be stored at 4–5 °C, whereas others require higher temperatures. At the Centre for Pacific Crops and Trees of the Secretariat of the Pacific Community (SPC), Fiji, plants are maintained at 20 °C or slightly above; the lower than normal temperatures slow growth, and rejuvenation is not a problem. This is the method recommended in this manual.

Notes



Mass propagation of plants: the Queensland experience

Chapters 5 and 6 showed how plants are tested for viruses after they have been given heat therapy and regrown from meristems. The next important step in our program is to multiply the plants that came through these tests without showing infection. They are apparently free from virus, and can be given to farmers. These activities conclude the pathogen-tested (PT) scheme.

Unfortunately, in many countries, especially those of the Pacific region, multiplying vegetatively propagated root crops and putting them in reach of farmers is no easy task. But farmers need good varieties, without pests and diseases. This means that they need PT varieties.

Sweetpotato is grown by hundreds of thousands of farmers in Melanesian countries, and some in Polynesia and Micronesia. In terms of food security, it is a very important crop, and replacing it would be very expensive—the value of the crop grown has been estimated to be many millions of dollars, in terms of the cost of importing a substitute food with equivalent calories. We need to ensure that farmers are getting the best yields possible, and that they are using planting material free from pests and diseases, particularly viruses, because of their association with yield decline.

At present, no PT schemes are established for any of the indigenous root crops in the Pacific islands, although the potential benefits of growing plants that are free from pests and diseases are well known. Therefore, this chapter describes the steps of the PT scheme at Gatton Research Facility (GRF), Queensland, which has been highly successful.

We focus on two important aspects: how the plants are multiplied to grow a crop, and how they are maintained to delay virus reinfection.

8.1 Establishment of plants in the screen house

Chapter 5 explained how plants could be regrown from meristems and later removed from tissue culture and placed in pots. These plants were then tested on *Ipomoea setosa*, and also by ELISA (enzyme-linked immunosorbent assay) (Chapter 6). The plants that passed these tests are the ones that we want to multiply. While virus tests are done on them, the plants are maintained securely in a screen house, glasshouse or screened compartment, to keep them free from insects. At GRF, plants that are successfully PT are placed back into tissue culture.

8.2 Multiplication of sweetpotato

Sweetpotato can be multiplied in insect-screened enclosures, if they are large enough, or in the field. If material is multiplied in the field, site selection is important. The site should **not** be near subsistence or commercial sweetpotato production areas, because of the possibility that the plants could become contaminated by pests, diseases and, in particular, viruses. The following describes the stages in the multiplication process in Queensland.

PT plants are taken from tissue culture each year and grown in a screen house (called an igloo). Vines from these plants are planted in the field to produce storage roots; these are then supplied to farmers, who plant them in nurseries to obtain sprouts for commercial production.

8.2.1 Propagating vines in the screen house

Propagation of vines in the screen house at GRF is illustrated in Figures 30, 31 and 32.

The propagation facility at GRF is a shade house, covered with solar weave and aphid-proof mesh. It has detachable plastic sides; when in place in the cooler months of the year, the plastic sides reduce air flow and increase temperatures.

Similar shade houses are now used in Papua New Guinea and Solomon Islands. They are slightly different in that the detachable plastic sides are not needed because of the higher temperatures compared with GRF.

At GRF, sweetpotato tissue culture plants are deflasked and grown in punnet trays in the igloo in early spring. They are planted 20 cm apart in tubs, drums and troughs filled with sterile media and with drain holes in the bottom. At first, the plants are watered daily by micro-irrigation; as the temperature increases and the plants grow, the frequency and duration of watering

increases, reaching a maximum in summer of 10 minutes twice a day.

Sticky traps are placed in the shade house and monitored weekly for the presence of insects and mites; they are changed every 3 months. The main pests in the igloos at GRF are mites during the summer months (from late November to April). Insects are also seen occasionally, presumably entering through doors or via holes in the solar weave, particularly those made during storms.

a. Plants from tissue culture grown in a punnet tray ready for transplanting

b. A 44-gallon plastic drum cut in half with drainage holes at the base, sufficient for five plants

c. A plastic feed trough, 1.5 m × 0.5 m, sufficient for about 15 plants

d. Plants in fibreglass tubs, 1 m × 1 m × 0.5 m, sufficient for 16 plants, with drippers

e. View of the igloo, showing fibreglass tubs, plastic drums and the irrigation system. The watering regime is on a timer, and is revised weekly as growth and temperature increase

f. Mother plants maintained in a screen house after indexing for viruses. Note the screened compartment at the back of the screen house

Figure 30
Establishing virus-indexed plants in the igloo at Gatton Research Facility

To eradicate insects and mites, the plants are sprayed every fortnight with appropriate pesticides, particularly those effective against whiteflies, aphids, caterpillars and mites.

Figure 31
Screen house (igloo) at Gatton Research Facility. Note that the entrance to the igloo (right) is through an insect exclusion room.

Figure 32
Screen house (igloo) at Gatton Research Facility showing sweetpotatoes in troughs 4 weeks after planting out. Note the large labels for each of the varieties, and yellow sticky traps to catch flying insects (left). The same plants at 8 weeks, ready for the first vine tip cutting. Depending on environmental conditions, vine tip cuttings are then made fortnightly (right).

The external walls of the shade house are checked regularly for holes; if damage is found, it is repaired immediately, and the plants inside are sprayed with pesticides for whiteflies, aphids, caterpillars and mites. Entry by people is kept to a minimum.

8.2.2 Cutting the vines

PT sweetpotato storage roots in Queensland are produced on sites (normally two) that are outside the commercial production areas. This reduces the chance that the PT plants will be reinfected by viruses spreading from nearby crops.

To produce the amount of commercial storage roots required each year, vines are taken from the shade house and planted in the field at GRF. This is done, on average, every 2 weeks from September to January. After about 8 weeks growth in the field, vines are taken from these field-grown plants and planted alongside cuttings taken from the shade house.

The steps, which are illustrated in Figure 33, are as follows:

- Vines, 35–40 cm long, are cut from plants in the shade house using scissors, knives or secateurs; this can be done every 2–3 weeks, depending on the growth rate.
- Cutting tools are sterilised between taking vines of different varieties.
- It is best to plant the vines within 24 hours of cutting them.
- If the vines are to be planted straight away, they are placed in a bucket with about 5 cm of water; this helps reduce the stress on the plants.
- The vines are not left in a bucket of water for more than 12 hours, since this would cause them to begin producing roots; these are delicate and easily damaged during planting, which has the potential to reduce yield.
- If the vines are to be held for more than 12 hours before planting, they are covered with damp hessian bags. This helps reduce stress due to water loss.

Figure 33
Healthy, vigorous plants grown in troughs in the screen house (igloo) at 10 weeks (left); they are now ready for a second cutting. The cut vines are about 35 cm long and placed in buckets containing 5 cm of water to keep them hydrated while they await planting in the field later the same day (right). The addition of chicken manure to soil or compost will produce vigorous plants.

- After the vines have been cut in the shade house, the 'mother' plants are trimmed and fertilised. In Papua New Guinea, the addition of chicken manure to the media has resulted in vigorous vine production.

! Terminal or tip cuttings are best. Back cuttings are only used if there are not enough terminal cuttings. Later, terminal cuttings are taken from these plants.

8.2.3 Planting the vines

The steps in planting the vines at GRF, illustrated in Figures 34, 35 and 36, are as follows:

- A soil analysis is always performed before planting to address any nutrient deficiencies or excesses in the field.
- Vines are planted as soon as they are ready in the shade house. The first vines from tissue culture plants are often

a. Vines taken from the screen house, 35–45 cm long

b. Newly cut vines laid out in the field ready for planting

c. From left to right: two rows at 4 weeks old, one row at 2 weeks old, and a part row ready for planting

d. Vines at 4 weeks (left), 2 weeks (centre) and 1 week (right)

e. Watering the vines—this is very important during the first week after planting

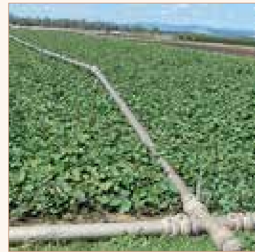

f. Vines at 3 months after planting

Figure 34
Planting the vines

spindly and tender; they snap easily, so must be handled with care when planting.

- The first lot of vines are planted 25 cm apart along the row; cuttings from these field-grown plants will be used to plant other rows. Later, vines are planted 35 cm apart.
- The vines are planted flat, with three to four nodes in the soil, and at a depth of 5 cm in the hotter months. Trials at GRF and elsewhere in Queensland on various planting methods have shown that this is the best way to plant sweetpotato vines. This way, the developing storage roots have more room to expand, resulting in less bending and twisting, and

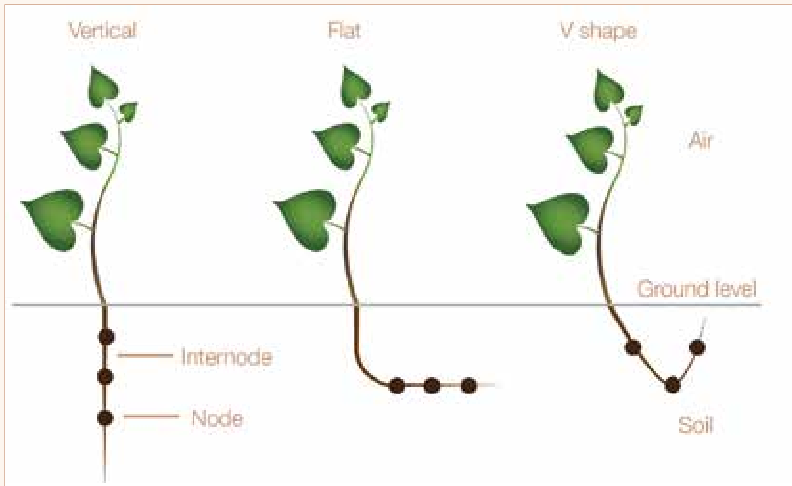


Figure 35
Different planting styles. Flat planting is preferred because it results in better root shape, as long as the vines are not shallow. If they are, and temperatures are high, yields may be reduced.

Figure 36
Roots from sweetpotato variety Beauregard. Note the more even shape and longer roots from the vine planted horizontally (left), compared with the vine planted vertically (right). The two planting methods are shown diagrammatically in Figure 35.

the roots are more uniform than when the vines are planted vertically.

- Different varieties are planted in separate blocks, because some varieties are more vigorous and tend to suppress others.

- A plan is kept of the varieties, along with all the planting dates, so that times of harvest can be calculated.
- Newly planted vines are watered immediately after planting and then daily for the first week. The vines from the screen house are soft and tender and need to be kept well hydrated to survive under field conditions. Overhead sprinkler irrigation is used for the first few weeks until the plants are established; plants are then watered by trickle tape irrigation.
- Tensiometers are used to determine water requirements (Figure 37).
- Plants are monitored regularly for insect damage and leaf abnormalities. Plants that look different from the others, and might be diseased, are removed.
- Sweetpotato weevil traps with pheromone baits are placed around the sweetpotato fields to monitor weevil activity (Figure 38). If weevils are caught in the traps, a spray program (currently pyrethroid insecticides) is carried out at 21–30-day intervals to control adult weevils and the new generations emerging from the roots.
- Wireworms (*Agrypnus* and *Gonocephalum* species, true and false wireworms, respectively) are monitored by cutting sweetpotato roots into pieces about 5–7 cm square, and burying them around the perimeter of the planting, about 1 m away from plants and 30–50 m apart. They are dug up every fortnight to check for wireworms or evidence of their feeding. Generally, preventive insecticides are incorporated into the soil before planting if wireworms are likely to be present at the site. Alternatively, multiple applications of insecticide are applied by trickle irrigation to minimise feeding injury to plants by wireworms.
- Levels of root-knot nematodes are assessed before planting. Approximately 10 g of soil is collected to a depth of 10 cm using a soil auger from locations around the planned cropping area. These samples are mixed together, and 300 g is taken for analysis. If root-knot nematodes are detected, a nematicide is used to prevent damage to the storage roots.

Figure 37
Tensiometers at different depths in the soil to measure water needs

Figure 38
A sweetpotato weevil trap with pheromone bait (the pheromone is absorbed onto the rubber tubing at the top of the trap)

- Nitrogen is carefully managed, because high levels can reduce yields of storage roots. The soil analysis is used as a guide. If needed, nitrogen is applied in split applications to maximise its uptake by the plants, and to reduce leaching of the nutrient from the soil into the environment, particularly river systems.
- Weeds are kept to a minimum since they can be hosts for viruses, as well as competing with sweetpotatoes for nutrients.

! Vines less than 30 cm and more than 45 cm long are slower to establish, and storage roots gain weight more slowly.

Flat planting can improve root shape in some soils; however, shallow flat planting may lead to lower numbers of marketable storage roots under hot growing conditions.

Vines are watered immediately after planting, and the soil is kept wet for the first week to ensure that the emerging roots develop their potential in terms of shape and yield.

In the case of the variety Beauregard, the early development of the adventitious roots (long white roots that develop from the nodes) is critical, because these will become the first marketable storage roots.

8.3 Harvesting and packing the storage roots

When the storage roots in the field nursery are ready, they are harvested and packed for distribution to growers, who set up their own nurseries for producing vines for commercial plantings.

The steps involved in harvesting, cleaning and packaging the storage roots, illustrated in Figures 39 and 40, are as follows:

- The vine tops are removed by slashing or 'top-chopping' them near the soil surface. Care is taken to avoid damage to exposed storage roots.
- If rain is expected, a few centimetres of vine are left, as this will use water and help dry out the soil around the storage roots, facilitating harvesting.
- Storage roots are left in the soil for 2 weeks to 'harden up', so that the skin is less prone to damage.
- A mechanical sweetpotato digger is used to harvest the roots.
- The roots are handled carefully to avoid damaging the skin; if they are not hardened properly, the damaged areas will become sunken and dark during storage, and may become sites for entry of bacteria and fungi during washing.
- Any misshapen storage roots are removed.

- The roots are cured by placing them in a shed under a tarpaulin for 4–5 days at high humidity. This allows any small wounds caused at harvest to heal.
- After curing, the roots are washed for 30 minutes in water containing 30 parts per million (ppm) of chlorine, to disinfect their surfaces and loosen the soil on them.
- The water tank for soaking the roots is emptied and refilled with fresh water every 2–3 hours, as the available chlorine is depleted by the soil and debris.
- The roots are washed with a small mechanical washer.
- Clean roots are sprayed with fungicide (currently a product in the benzimidazole group) as they exit the washer, to prevent rots during storage.
- Wet roots are allowed to drain, and are then stored in a shed ready for packing. Packing is usually done in the middle of winter (July) in Australia.
- Alternatively, the storage roots are kept in a cold room at 14 °C and 85% relative humidity (if held above 14 °C, roots will start to sprout).
- Roots are then packed and sent to growers on receipt of payment.

To produce water containing the correct concentration (in ppm) of chlorine, for soaking the storage roots, a little household bleach is added to the tank, and chlorine test strips are used to measure the amount of available chlorine. These strips are obtained from pool or agricultural supply stores.

Only clean tank rainwater or mains water is used for soaking and washing the storage roots, to avoid bacterial or fungal contamination, and potential rotting.

In some countries, vines are distributed to growers instead of storage roots. This is not done in Australia, since there is a much greater chance that pests and diseases are carried in the vine. Also, vines must be kept

hydrated and then planted within 24 hours; this is difficult when they have to be sent long distances. Roots can be sent long distances and then stored in sheds until growers are ready to plant them, whereas vines cannot.

a. A mechanical digger

b. Collecting the harvest

c. Putting the harvest into field bins

d. The storage roots tipped carefully into field bin, with soil attached

e. Unwashed storage roots in field bins covered with tarpaulins to cure them at high humidity

f. Storage roots in field bins curing in the shed

Figure 39
Harvesting and curing the storage roots

a. Soaking the roots in water with 30 ppm of chlorine for 30 minutes

b. A small mechanical washer, which also delivers fungicide before the roots are drained

c. Roots drying in the sun after washing

d. PT roots packed in half-tonne cardboard bins

e. PT roots packed in large plastic bins

f. PT Beauregard

g. PT Northern Star

h. A smaller consignment of PT Beauregard ready to be sent to a grower

ppm = parts per million; PT = pathogen tested

Figure 40
Cleaning and packaging the storage roots

8.4 Producing vines on-farm from PT storage roots

Farmers use the storage roots they receive as a source of planting material for their commercial operations. The storage roots are grown in nurseries, known as seed beds, and vines are taken from the seed beds to produce a crop.

The steps in the production of vines on-farm, illustrated in Figure 41, are as follows:

- Sweetpotato roots are stored in a dark, cool place (e.g. in a shed) before planting.
- An area for planting is selected away from other plantings of sweetpotato, to minimise the risk of virus infection.
- Windbreaks of taller crops such as corn or tall grasses are planted around the seed beds to reduce invasions of insect vectors of sweetpotato viruses, especially aphids and whiteflies.
- Roots are sprayed with fungicide (a product in the benzimidazole group) before planting.
- Roots are planted side by side in a raised bed, with a small (1–2 cm) gap between them. They are then covered with 3–5 cm soil, before being watered.
- In cooler areas, or in early spring, seed beds are covered with a plastic tunnel to increase soil temperature and promote early sprouting of the roots. When air temperature outside the tunnel reaches 28 °C, raise the end flaps of the plastic tunnel to increase air flow and lower the inside tunnel temperature. Lower the flaps as cooler evening/night temperatures return. Later, when night temperatures increase, the covers may be removed completely.
- Sprouted roots are watered regularly, fertilised, monitored for pests and diseases, and sprayed with pesticides, if necessary. Any sprouts that look different, especially those with small leaves or with net-like patterns on the leaves

a. A seed bed of PT storage roots. Note that the roots are not touching each other, to prevent the spread of rots, and that grass windbreaks are present on one windward side, to reduce infestations of insects

b. Seed beds covered by plastic tunnels

c. Seed beds with plastic tunnels removed to show the lush growth of vines

d. Seed beds of a number of different varieties

e. Taking vines from the seed beds to plant in the field

f. Bundles of vines of different varieties ready for field planting

g. The seed bed after cutting a single plot

h. The plots after all have been cut

PT= pathogen tested

Figure 41
Growing vines from PT storage roots

(Figure 1), which may be symptoms of virus infection, are removed immediately.

- Vines are cut, using a sharp knife or scissors, for commercial plantings when they reach 35–45 cm. Cutting the vines in this way encourages the production of more shoots. Cutting is done even if it is too early to establish commercial fields, in which case the vines are discarded.

Take care not to overwater the roots, or they may rot.

Plastic covers (tunnels) encourage shoot development from the storage roots in the seed bed in winter; however, once the outside air temperature reaches 28 °C, the plastic covers are removed during the daytime to reduce soil temperatures.

8.5 Using PT roots as planting material

The Australian PT sweetpotato seed scheme has been a success, and all sweetpotato farmers in Australia now use PT roots as a source of planting material. The returns to growers in terms of yield are considerable. The scheme is shown in Figure 42.

The highly successful GRF-developed system has shown what can be done to grow sweetpotato crops free from infection. Although it is not yet possible to implement every aspect of this advanced Australian scheme in Pacific island countries, it provides a gold standard to aim for, and shows conclusively that the PT approach is definitely the way forward.

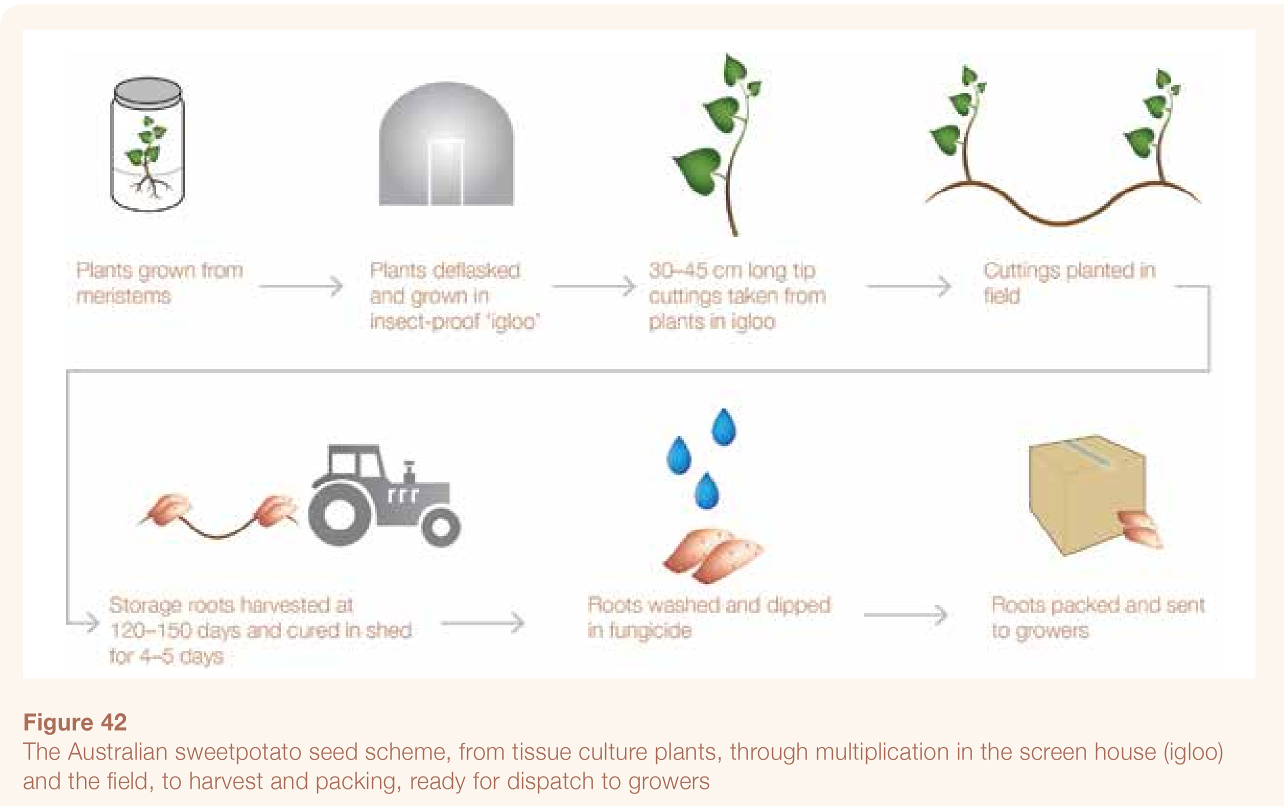
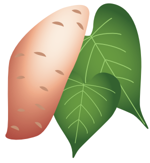


Figure 42
The Australian sweetpotato seed scheme, from tissue culture plants, through multiplication in the screen house (igloo) and the field, to harvest and packing, ready for dispatch to growers

Notes



Appendixes

Appendix 1
Formula for calculating bleach concentration

The formula to use when diluting bleach is:

concentration (original) × volume (original) =
concentration (final) × volume (final)

or

$C1V1 = C2V2$

where: C1 is the concentration of the bleach (sodium hypochlorite) as labelled on the bleach container, V1 is the volume of the bleach, C2 is the concentration you want (e.g. 1%, 1.5%, 2%, 2.5%, 3%) and V2 is the total volume you want to make.

For example:

C1 = 3.5%

V1 = volume of bleach to add; this is the unknown to be calculated (X)

C2 = final desired concentration is 1.5%

V2 = final desired volume is 1 L (1000 mL)

Calculation:

$C1V1 = C2V2$

$3.5 \times X = 1.5 \times 1000$

$X = 1.5 \times 1000/3.5$

$X = 429$ mL

Water is then added to 429 mL of bleach until the volume is 1 L, to produce a 1.5% sodium hypochlorite solution.

Appendix 2
Potting mixes: Australia and Papua New Guinea

Gatton Research Facility, Queensland

A modified University of California potting mix is used, called Qmix or Queensland Mix. It has sufficient fertiliser to keep the plants growing for about 6 weeks; after that time, a liquid fertiliser is applied weekly. The mix is pasteurised before use.

The Queensland mix has the following composition:

- Sand 40 L
- Peat 40 L
- Perlite 20 L

To 100 L of the above ingredients, the following nutrients are added:

Nutrient	Amount
Blood and bone	133.3 g
Dolomite	400.0 g
Hydrated lime	200.0 g
Potassium nitrate	13.3 g
Potassium sulfate	13.3 g
Superphosphate	133.3 g
Micromax[2] (trace elements)	33.3 g

2 Micromax is a commercial product containing all necessary trace elements for plant growth. It is said to be slowly available to plants over an 18-month period. It is likely that other commercial products would suffice for growing sweetpotato vine cuttings or storage roots.

Highlands Regional Research Centre, Papua New Guinea

The Papua New Guinea mix has the following composition:

- Black soil
- Chicken manure
- River sand

These ingredients are combined in the ratio 3:2:1 by volume—for example, 3 buckets of black soil, 2 buckets of chicken manure, and 1 bucket of river sand. The mix is pasteurised before use.

Appendix 3
Personal protective equipment

The Queensland Government defines personal protective equipment (PPE) as any clothing, equipment or substance designed to protect a person from risks of injury or illness. Details are given on the website of Workplace Health and Safety Queensland (<www.deir.qld.gov.au/workplace/subjects/ppe/whatisppe/index.htm>).

In our situation, working in the laboratory or field, and dealing with sweetpotato, PPE can include:

- clothing, such as lab coats
- surgical gloves
- eye and face protection, such as goggles or masks
- sun hats
- safety equipment for the application of pesticides
    - overalls
    - gumboots or other water-resistant footwear
    - respirators
    - rubber gloves.

Appendix 4
Best practice for the screen house environment

In the context of sweetpotato pathogen testing, a screen house is a facility designed to minimise the entry of insects that may carry virus to the plants inside. Plants grown in screen houses should be healthy and free from insect and mite infestations, as well as infections from bacteria and fungi. It is important that the screen house is kept clean. A number of problems occur if cleanliness is not maintained: insect infestations may spread viruses and diseases; contamination with fungi and bacteria make surface sterilisation and establishment in tissue culture difficult; and outbreaks of mites occur that damage plants and are often difficult to manage.

Screen house maintenance

General

- Screen houses should be located in an open space, away from trees and overhanging branches.
- Keep floors—whether they are concrete, gravel or soil—clean and free from weeds and algae.
- Remove old plants and vines as soon as possible—that is, plants in pots that are no longer in use, or any cuttings taken from the plants.
- Do not allow rubbish of any kind to accumulate in the screen house; do not use the screen house as a storage area.
- Factory-made sticky-backed felt strips, or cloth, can be wrapped around the frame where the supports meet the covering to reduce rubbing and the potential for tears.

- Regularly check all interior and exterior walls, doors and the roof for damage, especially after storms or strong winds.

Insects and mites

- If insects or mites are present, it is more likely that bacterial and/or fungal contamination will also be present; this makes surface sterilisation of the vines and establishment in tissue culture more difficult.
- Insect proof all places of potential entry, including windows, doors and walls.
- Regularly check for insects and mites.
- Spray fortnightly with insecticides; choose products that are active against mites as well as insects.
- Check that the quarantine-grade mesh or polyethylene fabric of the screen house is not rubbing on any parts of the metal or timber frame, especially on the corner joints, as rubbing can cause holes to form that may allow entry of insects and mites.

Bacteria and fungi

- Treat plants with a fungicide 1 week before taking shoot tips for culture.
- Always bottom-water pots.
- If air-conditioners or other air-cooling devices are used, clean the insides regularly to reduce bacterial and fungal growth.
- To reduce contamination when transfers are made to tissue culture medium, choose only healthy new growth.

Screen house repairs

From time to time, mesh-covered screen houses and tunnels will need repair. This is done as follows:

- Cut a piece of quarantine mesh or polyethylene fabric larger than the hole or damaged area to be repaired.
- Spread silicon (proprietary brands are available to fill gaps) around the hole or over the damaged area, and repair with the cut piece of mesh, making sure that there are no gaps where insects can enter.
- Check to see if the material needs to be clamped on the edges, using pegs or bulldog clips, until the silicon dries.

Appendix 5
Best practice for tissue culture laboratories[3]

The laboratory environment

- Keep the laboratory clean and uncluttered at all times.
- Have a separate area in the laboratory where dirty material is brought in for sterilisation—that is, do not allow dirty material into the area where tissue culture operations occur.
- Set aside a separate area in which to make up media.
- Keep perishable ingredients in a clean refrigerator or freezer that is restricted to tissue culture use only.
- Do all washing up as soon as possible and, preferably, away from areas used to make media.
- Place all waste into plastic bags, seal the bags, and remove them from the laboratory daily.
- Remove all contaminated tissue culture vials immediately, place them in sealed plastic bags, and autoclave them before disposal. If plants from contaminated vials are needed, deflask them into a pot and grow them in a shade house for reinitiating into tissue culture at a later date.
- Do not allow dust to accumulate on surfaces, walls, etc. Use household bleach to wipe down all surfaces.
- Use the UV lights in the laminar flow cabinets on a regular basis; put them on for 20 minutes before using the laminar flow cabinet, **but do not forget to turn them off before starting work**.

3 Provided by Jenny Moisander, formerly employed at the Redlands Research Facility, Cleveland, Queensland.

- Clean floors regularly—daily, if the laboratory has a high amount of use. Mop with a household bleach solution once a month.
- Wipe growth-room shelves every few months with household bleach; walls and ceilings can be done twice a year.
- Place insect traps (e.g. yellow sticky traps) around growth-room areas if insects are noticed.
- Use sticky mats on floors and entrances to clean rooms.
- Keep all personal protective equipment clean, and for laboratory use only:
    - Regularly wash lab coats.
    - Use disposable booties (cotton or paper foot covers), face masks and hair nets.
    - Do not reuse gloves.
- Consider the hygiene standards for everyone working in the tissue culture laboratory:
    - Use clean laboratory footwear. Preferably, have a pair of shoes that is only worn in the laboratory; otherwise, clean your shoes before entering the laboratory and place shoe covers over them while in the laboratory.
    - Have a clean set of clothes for laboratory work only.
    - On days when you are working in the laboratory, avoid doing any outside work before entering the laboratory.
    - If you have to work outside on the same day as working in the laboratory, shower and change your clothing before beginning laboratory work.
    - Wash your hands and arms with an antiseptic solution before beginning lab work.

Appendix 6
Multiplication and meristem medium recipe

To make up 1 L of medium

Redlands multiplication medium

MS 519[4]	4.43 g
Phytogel[5]	4.0 g
Sucrose	25.0 g

Distilled water to make up to 1 L

Method

Add ingredients to distilled water; dissolve by heating on a magnetic stirrer. Dispense 10, 30 or 40 mL lots into containers (see below). Any jars can be used, provided that they are autoclavable and have lids with a tight seal. Honey jars or jam jars are used at Redlands Research Facility and Gatton Research Facility.

Depending of the size of the culture bottle, dispense the medium as follows:

10 mL for 100 mL bottles

30 mL for 200 mL bottles

40 mL for 250 mL bottles

4 Sometimes referred to as M&S, MS 519 is a commercially available mix of nutrients that is suited to sweetpotato.

5 Phytogel is a gelling agent used as a substitute for agar; it has the advantage of being clear, which assists the detection of microbial contamination. Gelrite is another commonly used product.

Do not tighten the screw caps, allowing air flow. Autoclave for 20 minutes. Remove from autoclave; allow to cool; label with batch number, your initials and the date.

Redlands meristem medium

MS 519	4.43 g
6-Benzlaminopurine (BAP)	4.4 mL
Indole-3-acetic acid (IAA)	2.8 mL
Phytogel	4.0 g
Sucrose	25.0 g

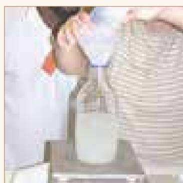
Distilled water to make up to 1 L

Method

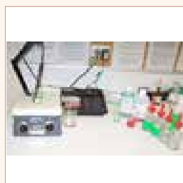
Add ingredients to distilled water; dissolve by heating on a magnetic stirrer. Dispense 10 mL lots of solution into 30 mL culture tubes; autoclave. Note that BAP and IAA are both made from commercial stock solutions (1 mg/mL).

Appendix 7
Making the media

a. MS 519 growth nutrients

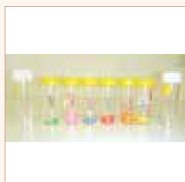


b. Adding sugar



c. pH testing equipment

d. Media on a magnetic stirrer hotplate



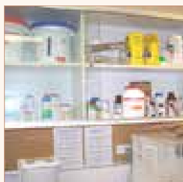
e. Coloured autoclavable (children's) beads used to identify the different types of media

f. Automatic media dispenser

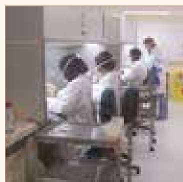
g. Jars—honey (left) and baby food (right)

h. Removing sterilised media from an autoclave. Note the use of personal protective equipment

i. Media ingredients and sterile water stored in a refrigerator



j. Separate storage area for non-perishable media ingredients



k. Working in laminar flow cabinets, and wearing appropriate personal protective equipment (masks, gloves and lab coats)

Figure A1
An illustrated guide to making tissue culture media

Appendix 8
Sweetpotato indexing record sheet

Sample ID	Grafting date	Symptoms recorded	ELISA date	FMV	LV	MMV	CFV	C-6	MSV	CaLV	CSV	SPVG	CMV

Appendix 9
Passport descriptors for sweetpotato[6]

1 Accession data

1.1 Accession number

This number serves as a unique identifier for each accession and is assigned by the curator when an accession is entered into the collection. Once assigned, this number should never be reassigned to another accession in the collection. Even if an accession is lost, its assigned number is still not available for reuse. Letters should be used before the number to identify the gene bank or national system (e.g. MG indicates an accession from the gene bank at Bari, Italy; PI indicates an accession with the United States system).

1.2 Donor name

Name of institution or individual responsible for donating the germplasm.

1.3 Donor identification number

Number assigned to the accession by the donor.

1.4 Other numbers associated with the accession

Any other identification number known to exist in other collections for this accession (e.g. USDA Plant Introduction number; not a collection number—see 2.1).

6 CIP, AVRDC and IBPGR 1991

1 Other number 1

2 Other number 2

1.5 Scientific name

1 Genus

2 Species

1.6 Pedigree

Parentage, or nomenclature and designations assigned to breeders' material.

1.7 Cultivar name

Either a registered or other formal cultivar designation given to the accession.

1.8 Acquisition date

Date on which the accession entered the collection (in the format DDMMYYYY).

1.9 Type of maintenance

Advanced cultivars, native cultivars and breeding lines with valuable gene combination should be maintained vegetatively:

1 vegetative in the field
2 vegetative in tissue culture
3 vegetative in the field and tissue culture
4 seed
5 vegetative in the field and seed
6 vegetative in tissue culture and seed
7 vegetative in the field plus tissue culture and seed

1.10 Date of last regeneration or multiplication

Date in the format DDMMYYYY.

1.11 Accession size

Approximate number of seeds of an accession in the gene bank.

1.12 Number of plants used in regeneration

2 Collection data

2.1 Collection number

Original number assigned by the collector(s) of the sample. It is normally composed of an abbreviation of the collector's last name(s), followed by a number. The collector's number is essential for identifying duplicates held in different collections. It should be unique and always accompany subsamples, wherever they are sent.

2.2 Collecting institute(s)

Institute(s) and/or people collecting or sponsoring the sample collection.

2.3 Date of collection of original sample

Date in the format DDMMYYYY.

2.4 Country of collection

Use the three-letter abbreviations supported by the Statistical Office of the United Nations. Copies of these abbreviations are available from the International Board for Plant Genetic Resources (IBPGR) and have been published in the FAO/IBPGR Plant Genetic Resources Newsletter, number 49.

2.5 Department/state

Name of the primary political subdivision of the country in which the sample was collected.

2.6 Province/county

Name of the secondary political subdivision of the country in which the sample was collected.

2.7 Collection site

Distance of the collection site in kilometres from the nearest town, village or map grid reference point (e.g. 15 km from Satipo, travelling to La Merced, Rio Negro).

2.8 Latitude of collection site

Degrees and minutes followed by N(orth) or S(outh).

2.9 Longitude of collection site

Degrees and minutes followed by W(est) or E(ast).

2.10 Altitude of collection site

Elevation above sea level (in metres).

2.11 Collection source

1 Wild habitat
2 Farmland
3 Farm store
4 Backyard
5 Village market
6 Commercial market
7 Institute
8 Other (specify in the 'Notes' descriptor, 2.16)

2.12 Type of sample

1 Storage roots
2 Stem cuttings
3 In vitro culture
4 Seed
5 Vegetative and seed

2.13 Status of sample

1 Wild
2 Weedy
3 Landrace/native cultivar
4 Breeder's line
5 Other (specify in the 'Notes' descriptor, 2.16)

2.14 Herbarium specimen

Was a herbarium specimen collected? If so, provide any identification number in the 'Notes' descriptor, 2.16.

0 No

+ Yes

2.15 Prevailing stresses

Information on associated biotic and abiotic stresses.

2.16 Notes

Some collectors will record ecological and soil information, cultural methods, months of planting and harvesting, uses of the plant, habitat of wild plants, etc.

Glossary

ambient temperature	The temperature of the surroundings.
amplifying	The result of PCR (polymerase chain reaction), whereby many copies are made of a nucleic acid segment (the target DNA).
antibodies	Proteins in blood that are produced in response to substances that are thought to be alien, such as bacteria, viruses and other foreign substances.
antisera (singular antiserum)	Blood serum (that is, blood without corpuscles or clotting factors), made in rabbits or other animals, containing antibodies that bind with specific antigens (e.g. viruses).
auger	A long, hollow tube with a spiral (corkscrew) inside for boring into the soil and taking a sample.
axillary bud	A young shoot at the junction of the stem and the leaf stalk.
blot	The initial step of ELISA testing where a leaf sap solution is applied to a nitrocellulose membrane. These membranes are stored (up to 6 months) until there are enough to economically run the complete ELISA.
chimeras	Patterns on a leaf, often lighter or darker than the rest of the leaf, caused by mutations. Because of the shades of green, they may look like virus infections.

cold light	Low-temperature light delivered through fibre optic sources; ideal for meristem dissection as it does not heat and dry the shoot tips.
cotyledons	First leaves produced by a seed.
degenerate primers	A mixture of similar, but not identical, primers that are used, for instance, where a gene is to be multiplied from different organisms.
ELISA	Enzyme-linked immunosorbent assay; a biochemical method for detecting proteins in a sample. The method is based on antigen–antibody interactions. In the sweetpotato ELISA test, the unknown antigen (virus) is fixed to a surface; a specific antibody is applied (10 antibodies in the CIP kit); an antibody to the first antibody is added with an enzyme attached; and this reacts with a (substrate) chemical, changing its colour.
esky	An Australian name for a brand of portable cool box, with two layers: polypropylene in the outer shell, and a polyurethane inner layer. This term is commonly used when referring to any such portable cool box.
gene bank	A collection of plant genetic material—as living plants, seed or frozen plant parts—that is used for conservation (i.e. preserving and storing the genetic material for future use).
germplasm	Genetic material that is transmitted from one generation to another.
grow light	Lights that emit at wavelengths that are beneficial for photosynthesis.

incubate	Keep the plants at a suitable temperature (usually 25 °C) so that they grow rapidly and normally.
in vitro	Process of growing plants in tissue culture in the laboratory.
meristem	A region at the growing tips of roots and stems of actively dividing cells, forming new tissues.
micro-irrigation	Low-pressure irrigation systems that spray, mist, sprinkle or drip.
necrosis	Death of cells of a leaf or other tissue as a result of disease or injury.
nitrocellulose membrane (NCM)	A membrane used as a support in diagnostic tests based on antigen–antibody reactions; the membrane is 'sticky'—that is, proteins (and nucleic acids) attach to it.
node	The part of the stem where a leaf is attached and where a bud resides.
nucleic acid	Chemicals in cells that are essential for life and occur in all organisms, made up of nucleotides. DNA and RNA are nucleic acids.
nucleotides	The building blocks of nucleic acids, such as DNA and RNA, composed of a phosphate group, a base and a sugar.
opaque	Something that cannot be seen through.

PCR	Polymerase chain reaction; a technique for making many copies of DNA segments. There are three steps: breaking the DNA apart, adding small pieces of nucleic acid called primers that bind to the DNA, and making a DNA copy using nucleotides, helped by the enzyme Taq polymerase. This is repeated to create many copies of the DNA segment.
petiole	The stalk of a leaf, attaching it to the stem.
phytoplasma	Small, specialised bacteria that live in the phloem of plants, and are transmitted by insects, most often leafhoppers. They are obligate parasites, meaning that they cannot live outside their hosts. They have cell membranes, rather than the rigid cell walls of typical bacteria.
primer	A small strand of nucleic acid (about 20 nucleotides) that matches the DNA (e.g. of a virus) that is being detected.
primordia (singular primordium)	Places on the meristem where leaves develop; the location of the primordia is genetically controlled.
probes	Similar to primers; small strands of nucleic acid that are specific to a species and labelled chemically so that they can be identified.
serology	The study of antibodies produced in blood serum for diagnostic purposes.
synthesis	The formation of new, more complex chemical compounds from two or more simpler ones.

Taq polymerase	An enzyme (originally from a bacterium), which works at high temperatures and is used during PCR. It helps to build new DNA, adding nucleotides to the DNA segment being copied.
terminal	At the end of something. In this manual, it refers to the meristem at the end of the shoot, which is sometimes called the apical bud.
vascular tissues	The phloem and xylem of plants—that is, the conducting tissues for plant nutrients and water. These tissues, with the vascular cambium, are also known as vascular bundles.
vectors	Organisms that transmit viruses; plant viruses are often spread by insects, but mites, fungi, nematodes and even some parasitic plants are also vectors.
veneer grafting	Usually a method for larger rootstocks, as the cut is deep and to the centre of the stem; the scion is wedge shaped, and inserted into the cut and held in place with tape.

References

Beetham P. and Mason A. 1992. Production of pathogen-tested sweet potato. ACIAR Technical Reports No. 21. Australian Centre for International Agricultural Research: Canberra. Accessible at <aciar.gov.au/publication/tr021>.

Bourke R.M. and Vlassak V. 2004. Estimates of food crop production in Papua New Guinea. Land Management Group, Australian National University: Canberra. Accessible at <ips.cap.anu.edu.au/ssgm/lmg/pubs/bourke-vlassak-crops.pdf>.

Bourke R.M., McGregor A., Allen M.G., Evans B.R., Mullen B.F., Pollard A.A., et al. 2005. Solomon Islands Smallholder Agriculture Study, volume 1. Australian Agency for International Development (AusAID): Canberra. Accessible at <ausaid.gov.au/Publications/Documents/solomon_study_vol1.pdf>.

CIP (International Potato Center), AVRDC (Asian Vegetable Research and Development Center) and IBPGR (International Board for Plant Genetic Resources) 1991. Descriptors for sweet potato, ed. by Z. Huamán. International Board for Plant Genetic Resources: Rome. Accessible at <sweetpotatoknowledge.org/germplasm/germplasm-collection/descriptors-for-sweet-potato>.

FAOSTAT 2007. FAO statistical databases. Food and Agriculture Organization of the United Nations: Rome. At <faostat.fao.org/site/339/default.aspx>, accessed 4 December 2012.

Loebenstein G., Thottappilly G., Fuentes S. and Cohen J. 2009. Virus and phytoplasma diseases. Pp. 105–134 in 'The sweetpotato', ed. by G. Loebenstein and G. Thottappilly. Springer Science+Business Media B.V. Accessible at <springerlink.com/content/j362425567010562>.

Further reading

Amante V.dR., Vasquez E.A., O'Sullivan J.N. and Norton G.A. 2003. A field guide to sweetpotato problems in the Philippines. University of Queensland: Brisbane.

Anon. 2010. Key access and utilization descriptors for sweet potato genetic resources. Bioversity International. At <www.cropwildrelatives.org/fileadmin/bioversity/publications/pdfs/1386_Key%20access%20and%20utilization%20descriptors%20for%20sweet%20potato%20genetic%20resources.pdf>, accessed 4 December 2012.

Bourke P.M. 2009. Sweetpotato in Oceania. Pp. 489–502 in 'The sweetpotato', ed. by G. Loebenstein and G. Thottappilly. Springer Science+Business Media B.V.

Bourke R.M. and Harwood T. (eds) 2009. Food and agriculture in Papua New Guinea. ANU E Press, Australian National University: Canberra. At <epress.anu.edu.au/titles/food_agriculture_citation>, accessed 4 December 2012.

Brunt A.A., Crabtree K., Dallwitz M.J., Gibbs A.J. and Watson L. (eds) 1996. Viruses of plants: descriptions and lists from the VIDE database. CAB International: Wallingford, Oxon, UK.

Clark C.A. and Moyer J.W. 1988. Compendium of sweetpotato diseases. American Phytopathological Society: Minnesota.

Clark C.A. and Hoy M.W. 2006. Effects of common viruses on yield and quality of Beauregard sweetpotato in Louisiana. Plant Disease 90, 83–88.

Coleman E.A., Hughes M.J., Jackson G.V.H., Komolong B. and Guaf E. 2009. Genetics and disease as factors in the yield decline of sweetpotato in the Papua New Guinea Highlands. Pp. 33–42 in 'Soil fertility in sweetpotato-based cropping systems in the highlands of Papua New Guinea', ed. by G. Kirchhof. ACIAR Technical Reports No. 71. Australian Centre for International Agricultural Research: Canberra. Accessible at <aciar.gov.au/publication/tr071>.

Drew R., Smith M. and Moisander J. 1991. Plant tissue culture: general principles and commercial applications. Information series. Queensland Department of Primary Industries: Brisbane.

Fuentes S. 2010. Sweetpotato virus indexing. PowerPoint presentation. Agricultural Research Center, Holetta, Ethiopia, 13–15 December. International Potato Center: Peru. At <sweetpotatoknowledge.org/seedsystem/training-communication-material/sp-virus-indexing-pdf>, accessed 4 December 2012.

Gao F., Gong Y. and Zhang P. 2000. Production and deployment of virus-free sweetpotato in China. Crop Protection 19, 105–111. Accessible at <sciencedirect.com/science/article/pii/S026121949900085X>.

Komolong B., Jackson G. and Coleman E. 2008. Sweetpotato pest and diseases: a literature review. Unpublished report for ACIAR CP/2004/071, 'Reducing pest and disease impact on yield in selected PNG sweet potato production systems'. Australian Centre for International Agricultural Research: Canberra.

Love S.L., Rhodes B.B. and Moyer J.W. 1987. Meristem-tip culture and virus indexing of sweetpotatoes. IBPGR Headquarters, Food and Agriculture Organization of the United Nations: Rome.

Moyer J.W. and Salazar L.F. 1989. Viruses and virus-like diseases of sweet potato. Plant Disease 73, 451–445. Accessible at <sweetpotatoknowledge.org/crop-management/deseases/viruses/Virus%20and%20Virus-like%20Disease%20of%20Sweetpotato.pdf>.

Moyer J.W., Jackson G.V.H. and Frison E.A. (eds) 1989. FAO/IBPGR technical guidelines for the safe movement of sweet potato germplasm. Food and Agriculture Organization of the United Nations: Rome/International Board for Plant Genetic Resources: Rome. Accessible at <sweetpotatoknowledge.org/seedsystem/training-communication-material/save-movement-of-sp-germplasm-pdf>.

O’Sullivan J.N., Asher C.J. and Blamey F.P.C. 1997. Nutrient disorders of sweet potato. ACIAR Monograph No. 48. Australian Centre for International Agricultural Research: Canberra. Accessible at <aciar.gov.au/publication/mn048>.

O’Sullivan J., Amante V., Norton G., van de Fliert E., Vasquez E. and Pardales J. 2004. Sweetpotato Diagnotes: a diagnostic key and information tool for sweetpotato problems. University of Queensland, Australian Centre for International Agricultural Research, International Potato Center, Philippine Rootcrops Research and Training Center. At <keys.lucidcentral.org/keys/sweetpotato/key/Sweetpotato%20Diagnotes/Media/Html/Introduction/Introduction.htm>, accessed 4 December 2012.

Pearson M.N., Keane P.J. and Thagalingham K. 1984. Little leaf: a disease of sweetpotato in Papua New Guinea probably caused by mycoplasma-like organisms. Phytopathologische Zeitschrift 109, 269–276.

Queensland Department of Agriculture, Fisheries and Forestry 2010. Sweetpotato. Queensland Department of Agriculture, Fisheries and Forestry: Brisbane. At <www.daff.qld.gov.au/26_14943.htm>, accessed 4 December 2012.

Taji A.M., Dodd W.A. and Williams R.R. 1997. Plant tissue culture practice, 3rd edition. University of New England: Armidale, NSW.

Van Wijmeersch P. 2001. The status of sweet potato variety evaluation in PNG and recommendations for further research. In ‘Food security for Papua New Guinea’, ed. by R.M. Bourke, M.G. Allen and J.G. Salisbury. Proceedings of the Papua New Guinea Food and Nutrition 2000 Conference, PNG University of Technology, Lae, 26–30 June 2000. ACIAR Proceedings No. 99, 674–682. Australian Centre for International Agricultural Research: Canberra.

Van Wijmeersch P. and Guaf E. 2010. Comparison of pathogen tested with non-pathogen tested planting material of 28 sweet potato varieties. National Agricultural Research Institute: Papua New Guinea.

Woolfe J.A. 1992. Sweet potato: an untapped food resource. Cambridge University Press: Cambridge.

Worthington M. 2009. Cryotherapy: a new method to eliminate pathogens from sweetpotato propagation materials. Powerpoint presentation. Graduate Group in Horticulture and Agronomy, University of California, Davis, California. At <www.plantsciences.ucdavis.edu/plantsciences_courses/hrt298/examples/HRT298_sweetpotato_presentation.pdf>, accessed 4 December 2012.

Yen D.E. 1974. The sweet potato and Oceania: an essay in ethnobotany. Bishop Museum Press: Honolulu.

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