Postflask management of cassava and yams

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Research Guide

69

International Institute of Tropical Agriculture

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Objectives

This guide is intended to enable you to:

- understand why tissue culture plantlets require special care
- handle and transplant tisssue culture plantlets
- construct basic structures for acclimatization of plantlets
- understand the procedures for acclimatization of cassava and yam tissue culture plantlets
- solve problems related to tissue culture plantlets

Contents

1 Tissue culture plantlets

Plant tissue culture is the propagation of plant cells, a group of cells, tissues, and organs in an artificial culture medium under aseptic conditions. Plants regenerated through this method are called in vitro plantlets or tissue culture plantlets. Plant tissue culture has a wide range of applications in agriculture. The most widely used application is pathogen elimination and micropropagation. This application is particularly important for vegetatively propagated crops such as cassava and yams. Disease infected plants could be cleaned and rapidly multiplied through tissue culture techniques for germplasm exchange across national boundaries.

The International Institute of Tropical Agriculture (IITA) through its germplasm collection, evaluation, and breeding efforts has selected/bred superior germplasm of cassava and yams which are resistant to major diseases and pests and have consumer acceptance qualities.

The selected germplasm is subjected to pathogen elimination through meristem culture. Plantlets regenerated from meristem culture are subjected to rigorous virus indexing and must be free from all known virus diseases that affect cassava and yams in Nigeria. They are inspected and certified by the plant quarantine authority in Nigeria. Each genotype should retain the characteristics of the donor plant from which the meristem was obtained.

Tissue culture plantlets are distributed to collaborators on request. Upon receipt, plantlets are acclimatized before they are transplanted in the field. Acclimatization of tissue culture plantlets is an important step in the production system.

However, once the plants are in the field, they may be infected by diseases, which occur in the area where they are planted.

2 Acclimatization of tissue culture plantlets

Postflask management refers to the acclimatization, hardening, or weaning of tissue culture plantlets after they are removed from culture containers. It is an important step in the transfer of tissue culture plantlets to field conditions. This is an intermediate stage (transition) between the culture containers and the field.

The main factors affecting the survival of these plantlets after they are transplanted from culture containers to the growing medium are:

- the moisture in the medium
- relative humidity
- light intensity
- temperature

The main differences between tissue culture plantlets and field/greenhouse grown plants are in the structure of the plant and the growing environment. A tissue culture plantlet has:

- less epicuticular wax
- widely open stomata
- poorly developed palisade layer in the leaf
- incomplete vascular connections between root and shoot

This makes the survival of tissue culture plantlets difficult once they are removed from culture containers and placed under natural conditions.

The net photosynthesis and carbon metabolism of tissue culture plantlets decrease after plantlets are transplanted

to soil. Full recovery is visually observed one week after transplanting. In vitro plantlets in culture containers are exposed to very high relative humidity, lower light intensity, optimal temperature, culture medium that provides optimum nutrient requirements, and aseptic conditions.

Acclimatization of micropropagated plantlets can start from in vitro by gradual reduction of relative humidity inside the culture container and exposure of cultures to higher light intensity. However, it is more common that plantlets are transplanted to high humidity conditions in an enclosed area/chamber, e.g., polyethylene tents or chambers under shade. The growing medium and container into which tissue culture plantlets are transplanted is important for good survival. Inhibitors or dramatic shifts in pH in a medium can adversely affect root growth and thus transplanting success. Sufficient porosity of the growing medium to allow adequate drainage and aeration is also important for good establishment. The inoculation of plantlets with mycorhyzae, the application of nutrient solutions, or a mild solution of fertilizer could also improve the plant vigor after transplanting.

3 Distribution package

Tissue culture plantlets are distributed in culture tubes with appropriate culture medium. Before packaging, the tissue culture plantlets are inspected visually and those that are contaminated with fungi or bacteria are removed. Cultures are packed in cardboard box(es) with:

- a phytosanitary certificate
- shipment form
- import permit (Fig. 1).

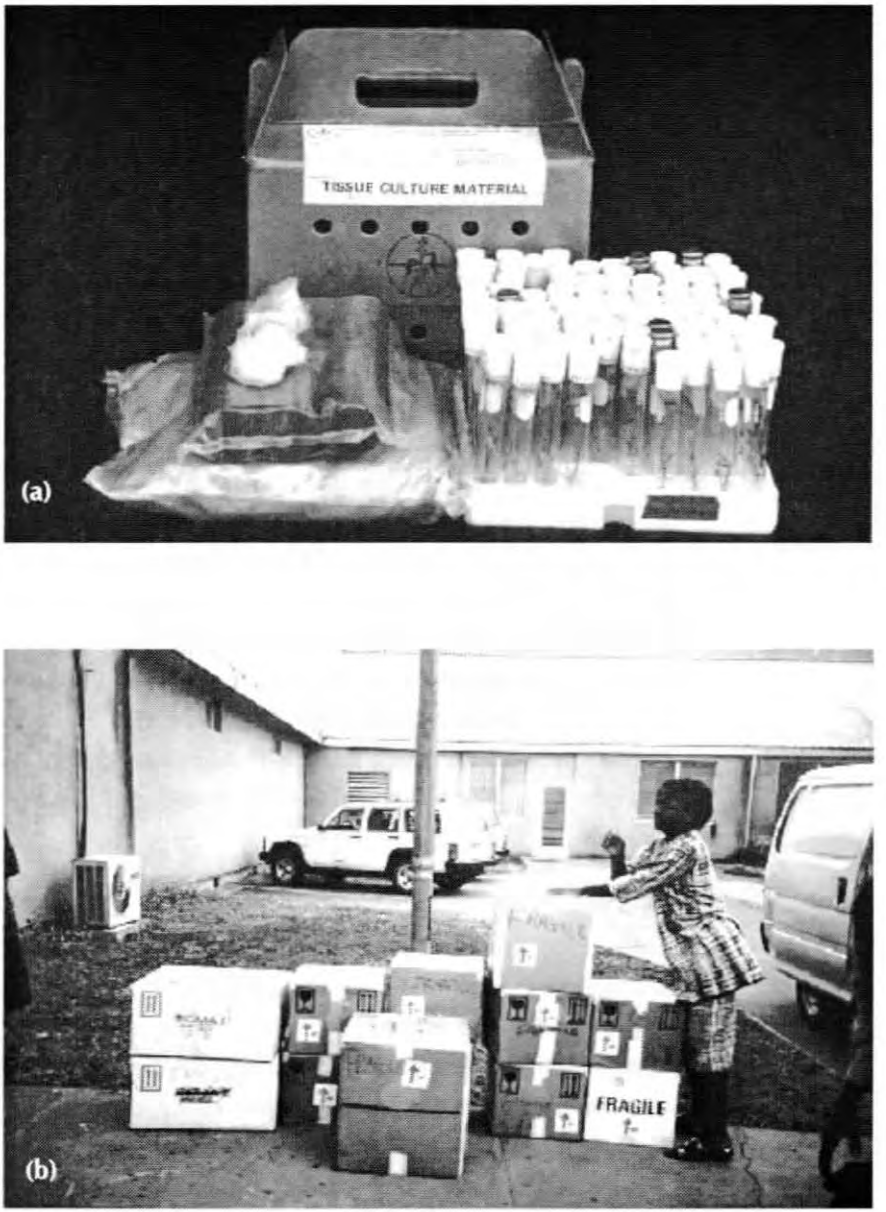

Figure 1. Distribution package of cassava and yam tissue culture plantlets: (a) small scale; (b) large scale.

Materials to be used for transplanting are packed separately. These materials include:

- jiffy peat pellets
- vermiculite
- plastic planting bags for cassava
- hand sprayer
- washing bottle

Some locally available materials such as coco-peat are suitable for use as planting medium. Coco-peat is produced by crushing the outer husk of a coconut. If coco-peat is used, it is autoclaved and cooled before use. Tissue culture plantlets and the materials for transplanting could be hand carried by air, or sent by courier services.

4 Handling during transport and upon arrival

Whenever possible, tissue culture plantlets are hand carried or sent as accompanying luggage to the destination to shorten the time in transit. Prolonged darkness results in deterioration of the plantlets, which in turn leads to low survival rate. Temperatures below 10 °C and above 40 °C must be avoided. Protect the package(s) from rain and prevent exposure to direct sunlight. Keep the box(es) in an upright position. If the journey is more than two to three days it is advisable to expose the cultures to light (not direct sunlight) whenever possible. If the package is checked in as accompanying luggage it must be marked fragile. The package must have clear "UP" and "FRAGILE" signs. The courier may take about five to seven days depending on custom formalities.

Upon arrival, plantlets should be transplanted as soon as possible. However, if this is not possible, it is advisable to unpack and place the cultures under sunlight or light

provided by fluorescent tubes and keep in an environment with temperature between 25 °C to 30 °C and away from dust.

5 Construction of basic structures for acclimatization of plantlets

Plantlets in culture medium are adapted to high relative humidity, almost 100%. Tissue culture plantlets usually have poorly developed cuticles, the stomata do not function properly, and the vascular connection between root and shoot may not be complete. These restrict water transportation from root to shoot at the same time increasing water loss. It is therefore important to provide an environment with high relative humidity.

A humidity chamber provides the right environment for tissue culture plantlets before transplanting. Plantlets are also grown in a culture room where light intensity is lower than sunlight. It is necessary that plantlets should be kept under low light intensity such as under a shade. Adequate water should be given. Temperature in the humidity chamber can range from 25 °C to 35 °C. Low temperatures could lead to poor plant growth in the humidity chamber and could lower plant establishment and reduce plant vigor.

Humidity chamber. Construct a humidity chamber with the following materials and follow the procedure outlined.

- Plywood board measuring 59 cm in diameter with four holes at equal length along the edge of the board. Each hole is about 1 cm in diameter. Locally available materials such as metal sheet, plastic covers, and basins can replace the plywood board.
- White glossy paint

- Plastic sheet (width 184 cm, length 125 cm)
- Masking tape
- Rope
- Stapler

Procedure

- If wooden boards are used, paint the boards with white glossy paint and allow them to dry well.

  Mark out 30 cm from the width of the plastic sheet.
- Run the plastic sheet along the edge of the wooden board and secure with staple pins as it goes along. Seal with masking tape at the joints and also at the base with the remaining plastic sheet. Secure the plastic sheet and the board by stapling.

Figure 2. Simple humidity chambers constructed using plywood and plastic sheet: (a) one layer; (b) multiple layers.

- The joint of the plastic sheet (longer end) is also sealed with masking tape. This upper portion is left open until after transplanting (Fig. 2).

A multilayer humidity chamber can also be prepared by connecting more than one sheet of plywood and hanging it on a rack made from bamboo (Fig. 2). If a plastic basin is used make sure that the basin is deep enough for the height of the plant with some allowance for growth. The basin can easily be covered with plastic sheet to maintain the high humidity.

Shading. The humidity chamber should be kept under shade and protected from animals and strong wind. The shade can be constructed with bamboo poles and the top covered with palm fronds to provide 60% shading. The humidity chamber can also be kept under the bench in a glasshouse, screenhouse, under natural shade or a big tree.

6 Procedure for acclimatization of cassava tissue culture plantlets

Use these materials and follow this procedure for the acclimatization of cassava tissue culture plantlets.

▶Jiffy peat pellets	Plastic planting bags of 9 cm x 3.5 cm x 5 cm
▶Vermiculite	Two buckets
▶Washing bottle	Hand sprayer
▶Mixing tray	Small, flat wooden stick
▶Scissors	Rope
▶Clean water	Marker pen
▶Labels	Fertilizer (NPK)

Procedure

- ▶Soak the jiffy peat pellets in one of the buckets with potable water about one hour before transplanting.
- ▶Remove the jiffy peat pellets from the bucket and at the same time remove the net from the pellet and break the peat moss into fine pieces in a mixing tray.
- ▶Mix two parts of peat with one part of vermiculite.
- ▶Label each plantlet to be transplanted indicating genotype and date of transplanting.
- ▶Fill each plastic planting bag with the mixture of vermiculite and peat up to half of the bag and arrange on a tray.
- ▶Fill the sprayer and washing bottle with potable water.
- ▶Remove the cover from the culture tube.
- ▶Use a small, flat wooden stick to loosen the edge of the culture medium from the culture container gently and carefully. Make sure not to break the shoot and the roots.
- ▶Hold the culture tube with the right hand (with the opening facing downward) and gently tap it against the left hand until the plantlet is half way out of the tube.
- ▶When the plantlet is out of the tube, do not hold its stem (because this will increase the possibility of breaking the whole root system from the stem). Allow the plantlet to rest on your palm. The culture medium normally remains attached to the root system (Fig. 3). To remove the culture medium, place your palm with the plantlet in water in the second bucket and shake gently to remove the medium.

- Place the plantlet in the half-filled plastic planting bag with the roots resting on the vermiculite and peat mixture. Add more of the mixture to cover the roots and base of the stem. Press the mixture gently to allow slight compacting. Insert the label and apply water to the mixture with washing bottle.
- Immediately after transplanting, place the plants in the humidity chamber.

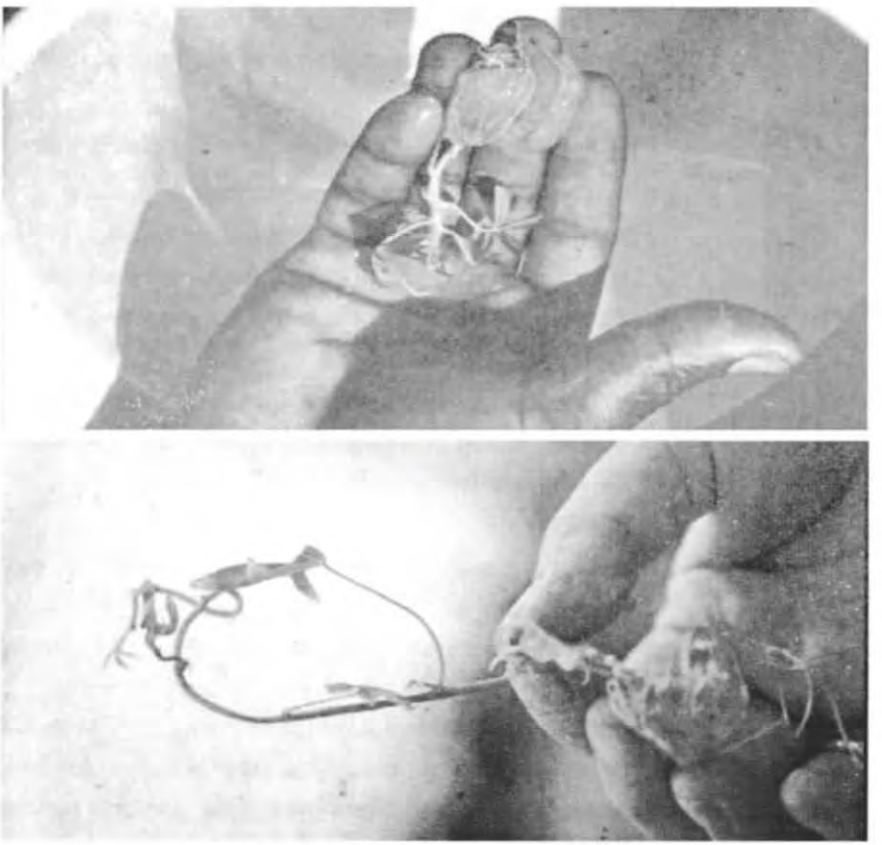

Figure 3. A cassava plantlet removed from the culture medim.

- Spray the humidity chamber generously with water and then close the top of the chamber by tying the plastic sheet with rope and securing it with the masking tape. Hang the humidity chamber in the shade by tying the end of the rope to the middle of the bamboo pole or bench support in a screenhouse (Fig. 4).
- 10 to 14 days after transplanting, puncture three little holes (about 1 cm in diameter each) at the sides of the humidity chamber using the tip of a pen.
- Two to three days later, reduce the humidity in the chamber further by cutting an opening (a half circle window of 14 cm diameter) at the lower side of the chamber. Fill the washing bottle with potable water and drop in about 6 to 8 grains of complete fertilizer (NPK). Mix very well. Water the plants with this solution.

Figure 4. Humidity chambers with tissue culture plantlets hang under shade.

Adequate amounts of water must be given but not excessively. Spray the humidity chamber to maintain humidity. Check the plants daily and water when necessary.

- Two days after cutting opening the first window, cut another window on the opposite side of the first window (Fig. 5). By now the humidity in the chamber is almost if not equivalent to ambient condition. Check and water the plants daily.

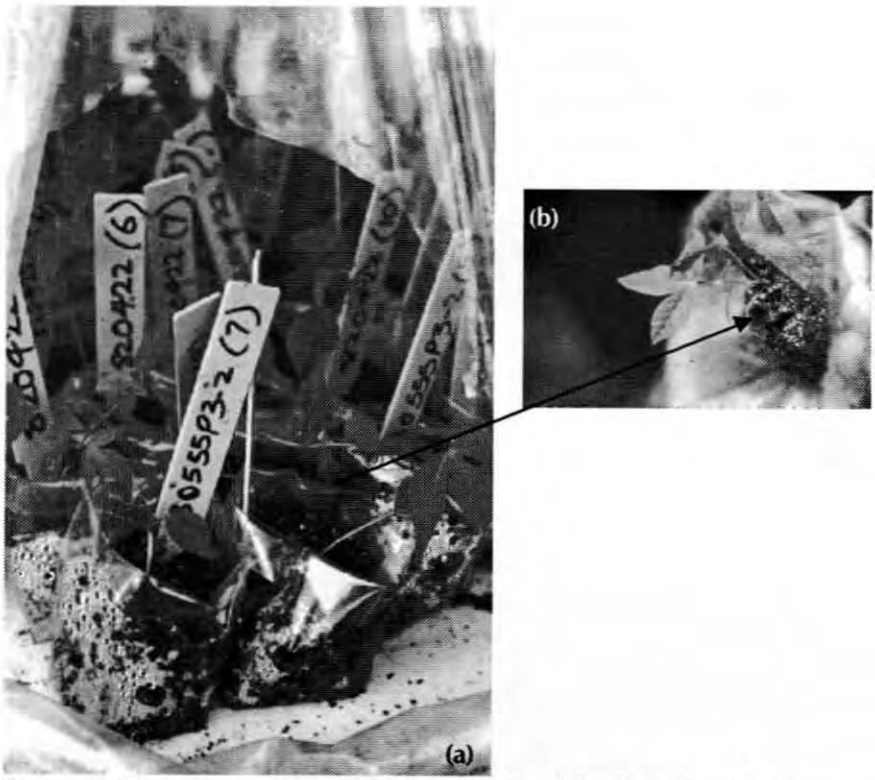

Figure 5. (a) An opening cut at the lower side of the humidity chamber; (b) Plant removed from humidity chamber ready for transplanting directly to seedbed or plastic polyethylene bag.

- At 21–24 days after transplanting, plants have formed new leaves and roots. Plants can remain in the chamber for another two to three weeks and be directly transplanted to a seedbed at 50 cm apart.

  You may also transplant to polyethylene bags filled with soil.

- For transplanting to polyethylene bags, remove plants from the humidity chamber and cut open one side of the planting bag. Transplant to soil in big polyethylene bag. After transplanting, place under shade and water regularly. Keep the plants under shade for another three to four weeks until they are about 15 cm tall (Fig. 6) when they are ready for transplanting to seedbed or field.

Figure 6. Cassava plants established in polyethylene bags after removing out from the humidity chamber

- Prepare the seedbed and dig holes at a spacing of 50 cm x 50 cm (Fig. 7). It is preferred to transplant early in the morning or late in the afternoon. Before transplanting, water the plants. Press the soil in the polyethylene bag gently to allow compacting, then cut open the polyethylene bag and transplant the whole lump of soil together with the plant to the hole and cover with soil (Fig. 7). Water the plants generously using a watering can. If the weather is very dry, plants will need to be watered twice a day. But if it is during the rainy season you may only need to water once a day or not at all. Sufficient water is required until plants are well established.
- Semi-hardwood cuttings can be obtained 6 to 8 months after transplanting to seedbeds.

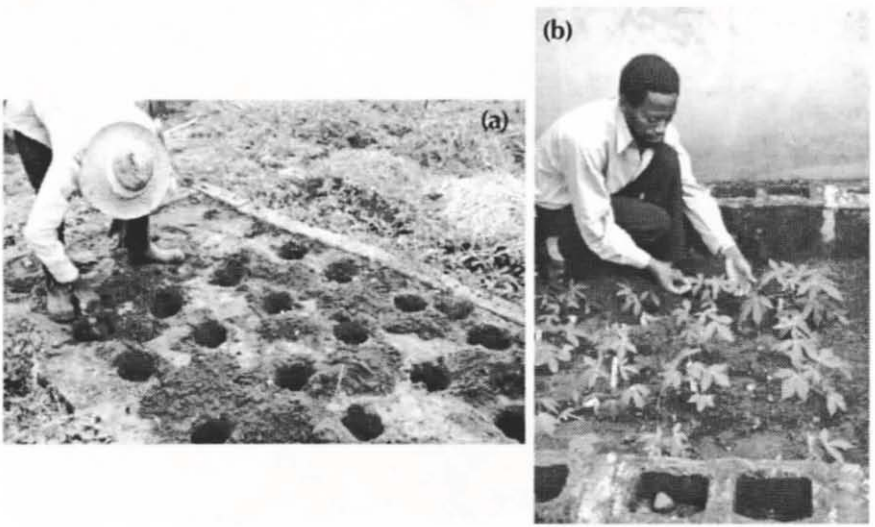

Figure 7. (a) Preparation of seedbed; (b) Cassava plants transplanted to seedbed.

7 Procedure for acclimatization of yam tissue culture plantlets

Use these materials and follow the procedure for the acclimatization of yam tissue culture plantlets.

- ▶Jiffy peat pellets (can be substituted with sterile coco-peat in plastic planting bags of 9 cm x 3.5 cm x 5 cm)
- ▶Two buckets
- ▶Hand sprayer
- ▶Scissors
- ▶Clean water
- Washing bottle
- Small, flat wooden stick
- Rope
- Fertilizer (NPK)

Procedure

- ▶Soak the jiffy peat pellets in a bucket of water an hour before transplanting.
- ▶When the peat pellets attain their final volume, remove them from the water and pierce a hole in each using a stick or the top of a marker pen. If coco-peat is used, sterilize in an autoclave at 121 °C for 1 hour, cool, and pour in plastic planting bags.
- ▶Write a label for each plant indicating genotype, number, and date of transplanting.
- ▶Remove cover from the culture tube.
- ▶Use a small, flat wooden stick to loosen the edge of the culture medium from the culture container very gently and carefully. Make sure not to break the shoot and the roots.
- ▶Hold the culture tube with the right hand (with the opening facing downward) and gently tap it against the left hand until the plantlet is half way out of the tube.

- When the plantlet is out of the tube, do not hold its stem (because this will increase the possibility of breaking the whole root system from the stem). Allow the plantlet to rest on your palm. The culture medium normally remains attached to the root system. To remove the culture medium, place your palm with the plantlet in the water of the second bucket and shake gently to remove the medium.
- Place the plantlets inside the hole of the jiffy peat pellet and press gently on the topside of the pellet to close the hole. Insert the label and place the plant inside the humidity chamber.
- For the rest of the procedure follow from step 12 under "Transplanting of cassava" except for transplanting to soil in polyethylene bag (step 17). In this step the net of the jiffy peat pellet is removed before transplanting to the polyethylene bag filled with soil. You may also transplant directly to the seedbed at 25 cm x 25 cm spacing.
- After transplanting to seedbeds/pots, carry out all agronomic practices such as weeding and staking (Fig. 8). At 6 to 8 months after transplanting to seedbed, plants will senenence and tubers can be harvested. Depending on the establishment of the plant, tubers in sizes varying from 5 g to 250 g can be obtained (Fig. 9).

8 Problems

Problems associated with tissue culture include:

- poor plant growth
- fungal attack
- insect attack

Figure 8. Yam tissue culture plants established in pots (a) or seedbeds (b) after acclimatization.

Poor plant growth. Before opening the humidity chamber, observe if the plants look healthy, if they have healthy green leaves and strong stems. If the plants show signs of weakness delay the opening of the chamber for a few more days up to one week. Poor growth often occurs when the ambient temperature is low (below 25 °C).

Fungal attack. There could be fungal attack due to the high humidity in the humidity chamber, which makes it an ideal environment for the growth of microbial organisms, especially fungi. If you spot any white spots forming in the planting mixture, consult a pathologist for advice. In the absence of a pathologist, add 500 mg of benalt to 1000 ml of solution, which is used for watering the plants (water with NPK).

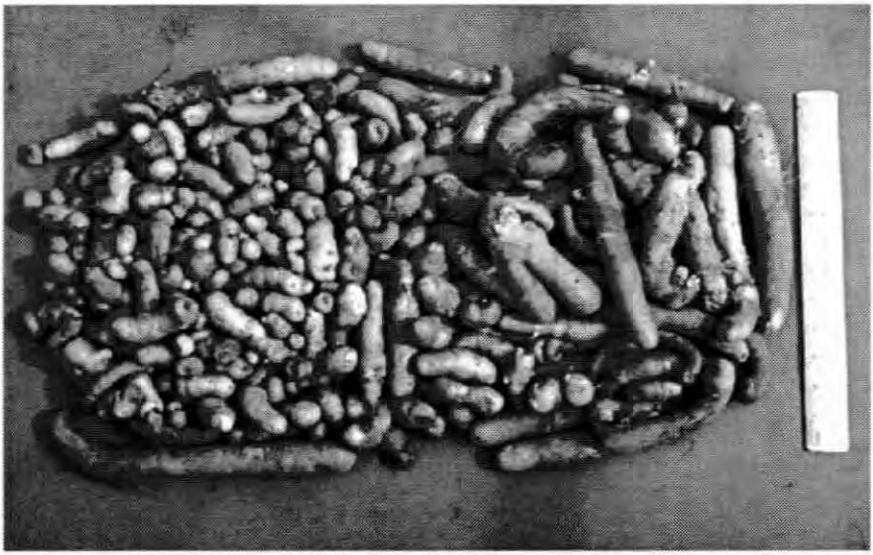

Figure 9. Yam minitubers harvested from tissue culture plants growing in seedbeds.

Insect attack. Insects especially caterpillars could cause serious problem. This happens only after the humidity chamber is open or the plants are transplanted to seedbeds. If not done carefully the leaves can all be consumed overnight. Consult an entomologist for advice and apply appropriate insecticides promptly.

9 Further reading

Dunstan, D.I. 1981. Transplantation and post-transplantation of micropropagated tree-fruit rootstocks. Combined Proceedings of International Plant Propagation Society 31: 39–45.

Dunstan, D.I. and K.E. Turner. 1984. The acclimatization of micropropagated plants. Pages 123–129 *in* Cell culture and somatic cell genetics of Plants Vol. 1. Laboratory procedures and their applications, edited by I.K. Vasil (ed.). Academic Press, Orlando, USA.

Griffis, J.L., Jr. G. Hennen, and R.P. Oglesby. 1983. Establishing tissue cultured plants in soil. Combined Proceedings of International Plant Propagation Society 33: 618–622.

McCown, D.D. 1986. Plug systems for micropropagules. Pages 53–60 *in* Tissue culture as a plant production system for horticultural crops, edited by R.H. Zimmerman, R.J. Griesbach, F.A. Hammerschlag, and R.H. Lawson. Martinus Nijhoff, Dordrecht, Germany.

Ng, S.Y.C. and R. Asiedu. Acclimatization of in vitro plantlets of white yam (*Dioscorea rotundata* Poir.): transition from culture tubes to field. Pages 435–438 *in* Proceedings of Sixth Triennial Symposium of the International Society of Tropical Root Crops-Africa Branch. ISTRC-AB/IITA.

Ng, S.Y.C., P. Ilona, and O.J. Adeniyi. 1994. Postflask management of cassava and yam. Tropical Root and Tuber Crop Bulletin 8(1): 6–7.

Preece, J.E. and E.G. Sutter. 1991. Acclimatization of micropropagated plants to the greenhouse and field. Pages 71–93 *in* Micropropagation-technologies and applications, edited by P.C. Debergh and R.H. Zimmerman. Kluwer Academic Publications, The Netherlands.

Van Huylenbroeck, J.M. and P.C. Debergh. 1996. Physiological aspects in acclimatization of micropropagated plantlets. Plant Tissue Culture and Biotechnology 2(3): 136–141.

Ziv, M. 1986. In vitro hardening and acclimatization of tissue culture plants. Pages 187–196 *in* Plant tissue culture and its agricultural applications, edited by L.A. Withers and P.G. Alderson. Butterworths, London, UK.

10 Suggestions for trainers

If you use this Research Guide in training:...

Generally:

- Distribute handouts (including this Research Guide) to trainees one or several days before your training activity, or distribute them at the end of your presentation.
- Do not distribute handouts at the beginning of a presentation, as trainees will read instead of listening to you.
- Ask trainees not to take notes, but to pay full attention to the training activity. Assure them that your handouts

(or this Research Guide) contain all relevant information.

- Keep your training activities practical. Reduce theory to the minimum that is necessary to follow the practical exercises.
- Use the list of questions which follows for testing. Allow consultation of handouts and books during examinations.
- Promote interaction of trainees. Allow questions, but do not deviate from the subject.
- Control your time.

11 Questions

1. What is postflask management?
2. Can you transplant in vitro plantlets directly to the field? Explain why.
3. Are in vitro plantlets different from plants propagated in the field? If so explain the differences.
4. What are the advantages of using in vitro plantlets?
5. What are the special features of the culture conditions of in vitro plantlets?
6. What are the main factors that affect the establishment of in vitro plantlets after transplanting from culture containers?
7. When removing in vitro plantlets from culture containers what special care must one exercise?
8. Can you suggest alternatives for maintaining high humidity conditions and alternative structures for humidity chamber using locally available materials?
9. Suggest alternative structures for providing shades?

10. Suggest alternate means for supplying adequate water to plants after humidity chamber opens?
11. What are the important characteristics of a substance, which will be suitable for use as medium for transplanting in vitro plantlets?
12. Suggest alternative substances which are locally available for use as transplanting medium?
13. Describe the procedures for the acclimatization of cassava in vitro plantlets?
14. Describe the procedures for the acclimatization of yam in vitro plantlets?
15. Explain why the humidity chamber is opened step by step opened?
16. What are the differences between the acclimatization of cassava and yams?
17. What alternative would you suggest if no potable water is available?
18. How would you overcome the low ambient temperature?
19. Will shading the plants after transplanting to seedbed be useful? Explain why.
20. How would you protect the humidity chamber from strong wind?
21. How would you protect the humidity chamber or young plants from attacks by animals such as cattle?
22. Will the application of rooting hormone promote in vitro plant establishment?

About IITA The International Institute of Tropical Agriculture (IITA) was founded in 1967 as an international agricultural research institute with a mandate for improving food production in the humid tropics and to develop sustainable production systems. It became the first African link in the worldwide network of agricultural research centers known as the Consultative Group on International Agricultural Research (CGIAR), formed in 1971.

IITA's mission is to enhance the food security, income, and well-being of resource-poor people primarily in the humid and subhumid zones of sub-Saharan Africa, by conducting research and related activities to increase agricultural production, improve food systems, and sustainably manage natural resources, in partnership with national and international stakeholders.To this end, IITA conducts research, germplasm conservation, training, and information exchange activities in partnership with regional bodies and national programs including universities, NGOs, and the private sector. The research agenda addresses crop improvement, plant health, and resource and crop management within a food systems framework and targetted at the identified needs of three major agroecological zones: the savannas, the humid forests, and the mid-altitudes. Research focuses on smallholder cropping and postharvest systems and on the following food crops: cassava, cowpea, maize, plantain and banana, soybean, and yam.

IITA Research Guides

IITA Research Guides are intended to widely disseminate information about all aspects of IITA's research, and especially new technologies. They are aimed at a broad audience mainly within sub-Saharan Africa, including agricultural researchers, trainers and trainees, and extension workers. For a complete list of IITA Research Guides, please contact IITA.

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