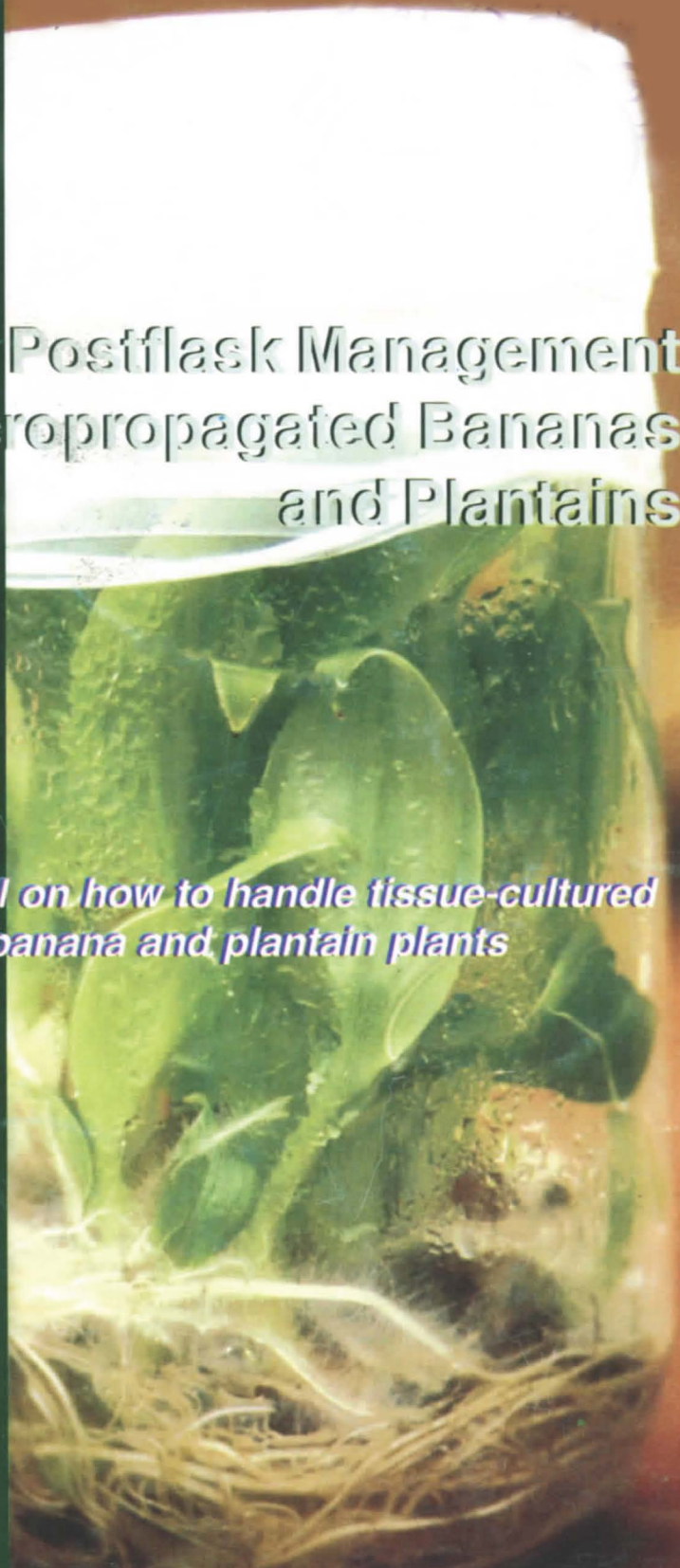




# Postflask Management of Micropropagated Bananas and Plantains

*A manual on how to handle tissue-cultured  
banana and plantain plants*





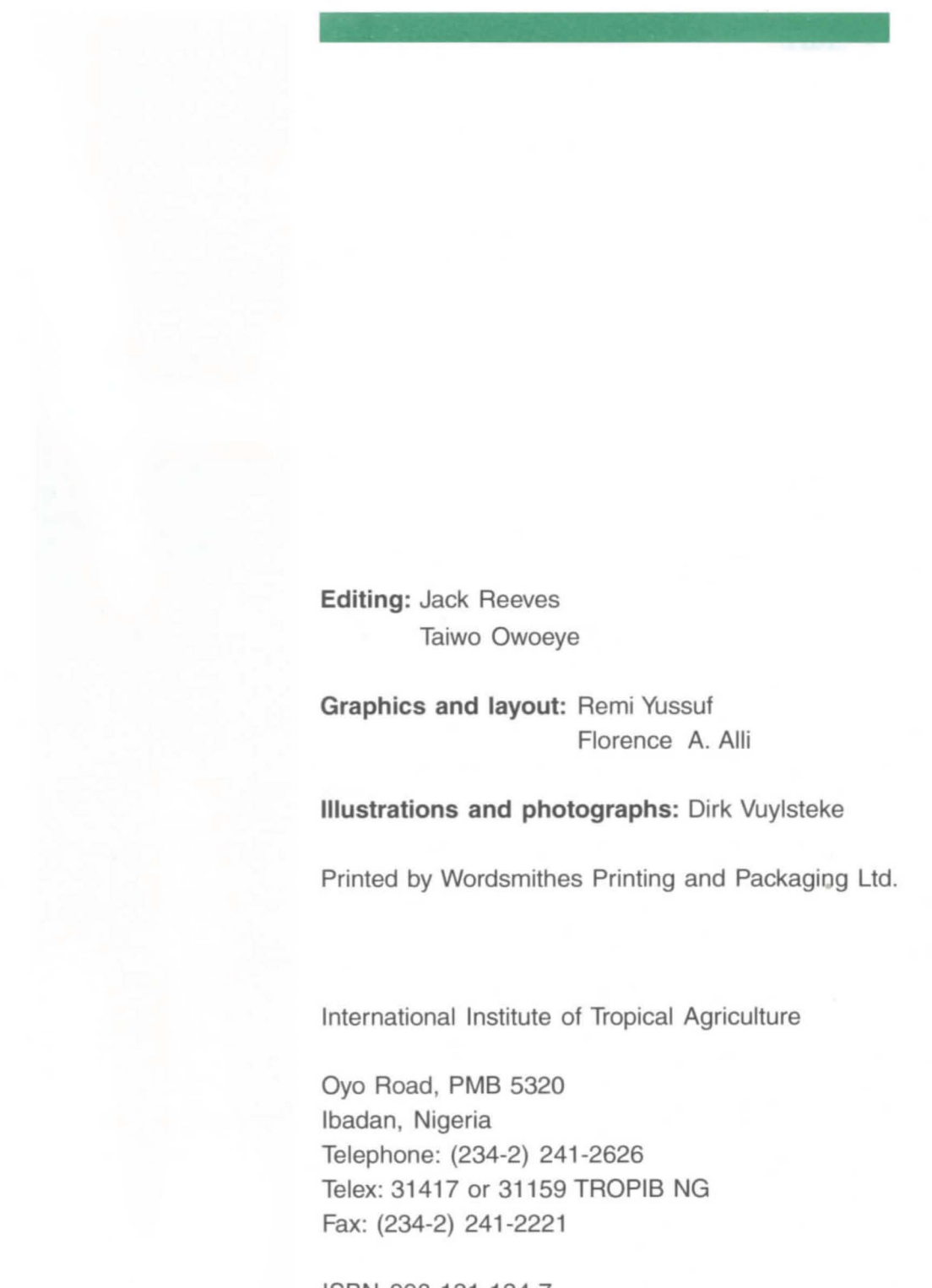
# Postflask Management of Micropropagated Bananas and Plantains

*A manual  
on how to handle tissue-cultured  
banana and plantain plants*

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## Foreword

Micropropagation techniques have the potential to deliver large amounts of propagules of vegetatively propagated plantain and banana, enabling the rapid dissemination of landraces and new varieties resulting from breeding programs. However, a critical stage in the realization of this potential is postflask management, in order to rear the in vitro plantlets to a stage where field establishment is assured.

This manual provides a stage-by-stage practical guide to postflask management beginning at the first stage of removal from the tissue culture facility through to the final stage of field establishment. The authors have drawn largely upon their own practical experience of this process, working in both lowland and mid-altitude humid environments.

It is intended that this manual will be used by the technicians who are practically involved in the dissemination of plantain and banana germplasm.

We hope it enables them to develop skilled green fingers.

**F.M. Quin**

*International Institute of Tropical Agriculture  
Ibadan, Nigeria*

## Introduction

Tissue culture as a means of banana and plantain propagation is increasingly being used by researchers and nursery personnel in both the public and private sector. The advantages of tissue culture micropropagation include higher rates of multiplication, production of clean or disease-free planting material, and the small amount of space required to multiply large numbers of plants. These advantages are particularly relevant to vegetatively propagated crops such as banana and plantain (*Musa* spp. L.), in which germplasm handling typically is fraught with such obstacles as slow multiplication, bulkiness, and poor phytosanitary quality of conventional propagules.

Tissue culture plays a vital role in the movement of germplasm and planting material between and within countries. It provides a tool for the safe international dissemination and propagation of newly selected cultivars or hybrids of banana and plantain.

Tissue culture can produce large numbers of healthy, clonal banana and plantain plants. However, tissue-cultured plants are more fragile than conventional propagules such as suckers. Successful establishment of these plants thus requires some care and special nursery conditions. Tissue-cultured plants are produced in a closed, sterile environment and grown in a nutrient-rich artificial medium under controlled conditions. When removed from the tissue-culture environment, micropropagated plants must be allowed to adjust to the outside environment with its varying light levels, changing temperature, reduced humidity, lower nutrient availability, and pathogen presence. This acclimatization is achieved during a hardening phase in the nursery.

This manual describes the procedures for successfully handling micropropagated bananas and plantains from flask to field.

## Handling

### During Transport

Whenever possible, tissue-cultured plants should be hand-carried from the place of production to destination. This usually shortens time in transit. However, tissue cultures can be successfully transported using regular or courier mail, if properly prepared and packaged. Prolonged periods in darkness, such as in a box used for transport, may result in weaker plants and a lower survival rate. Temperatures below 10 °C and above 40 °C should be avoided. Packages containing flasks with tissue-cultured plants should be protected from rain and direct sunlight and, if possible, kept in an upright position. If the transport period exceeds two days, it is advisable to occasionally expose the flasks to light, if possible.

### Upon Arrival

Tissue-cultured plantlets should be taken to the nursery as soon as possible upon arrival. If this is not possible, the flasks should be removed from their package or box and placed in a relatively clean room with indirect sunlight or fluorescent light. A room temperature of 20–35 °C is adequate. Micropropagated plantlets that are ready for deflasking can usually be kept in the flask for another 2–4 weeks.

Flasks should be monitored for microbial contamination for 1–2 weeks and those infected should be destroyed by the plant quarantine or crop protection service.

## Deflasking

Micropropagated plants can be deflasked at various sizes, but best results are achieved when plantlets are 7–10 cm tall and have a strong root system (Plate 1). Deflasking should be done out of direct sunlight in a sheltered, shaded area.

### Potting mixture preparation

A potting mixture is required for transplanting the plantlets. This should be prepared in advance. Top soil mixed with composted organic matter is recommended as an improved nursery substrate. Examples are (a) top soil mixed with dry cow manure at a ratio of 12:1 or (b) top soil mixed with palm fruit pulp and dry chicken manure at 7:2:1. Other potting mixtures can be made from locally available materials.

The soil or soil mixture may be pasteurized or sterilized to keep the plantlets free from soilborne pests and diseases during the nursery stage, but this is not always necessary or feasible. Pasteurization of the soil is done by steaming. An old oil drum, modified by welding iron cross bars at about 20 cm from the bottom, can be used for steaming. The drum should be placed on a fire place and 10 liters of water poured into it. The cross bars should be covered with a gunny bag to prevent the soil from falling into the water. The drum should be filled with the soil or potting mixture and covered with another gunny bag to prevent the steam from escaping too quickly. The drum should be fired

### Step 1



Plate 1

*Tissue-cultured banana plantlets ready for deflasking*



until steam has passed through the soil for about 1 hour. After pasteurization, the soil should be allowed to cool, which takes 1 day, and kept for at least 2 days before use.

## Step 2

### Pot filling

Nursery containers should be filled with the potting mixture. The preferred containers are 3-liter black polyethylene bags (about 15 cm diameter, 20 cm high), but any other available containers can be used. In case a humidity chamber stage (see Step 6a) is included in the nursery procedure, small plastic pots (5 cm diameter, either transparent or black) should be used and filled with the potting mixture before the larger black polyethylene bags are prepared and used.

## Step 3

### Plantlet removal

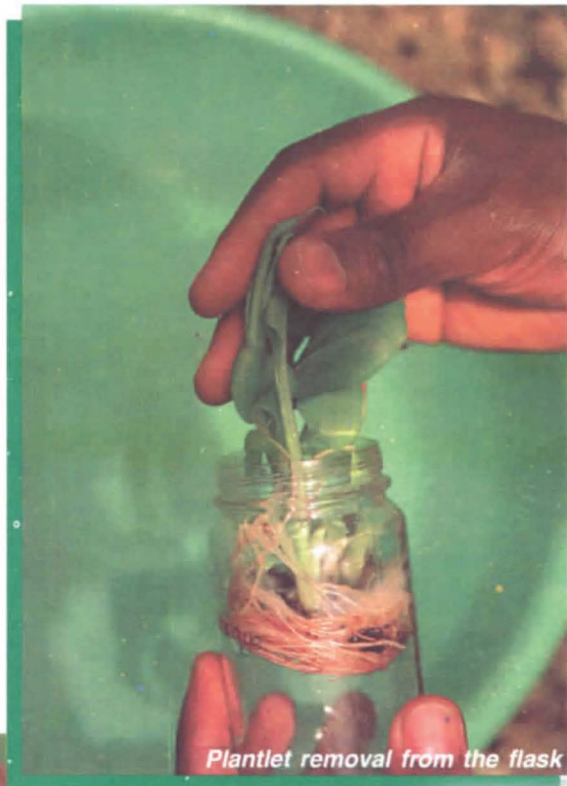
The flasks that contain the tissue-cultured plants should be opened by removing the cap. In case the plantlets were received in special tissue-culture bags, scissors can be used to open the bags at the top. Water can be added to the flask, which should then be gently shaken to loosen the agar and plantlets, if these stick to the bottom of the flask.

The plantlets should be carefully pulled out of the tissue-culture flask, using either forceps or fingers (Plate 2), and placed in a basin or bucket of water. The agar or nutrient medium should be gently washed off the roots (Plate 3) to avoid pathogen infection of the root system. If a cluster of plantlets was produced in the tissue-culture flask, these can be separated into individual plants by gently pulling them apart at the base or by a careful scalpel cut through the basal corm tissue. Long roots should be carefully disentangled and trimmed back to about 1–2 cm to ease planting.

## Step 4

### Plantlet transplantation

Individual plantlets should be transplanted into the potting mixture in the appropriate container (see Step 2). Care should be taken not to damage the short, fragile roots during planting. A small planting hole (about 2 cm deep) should be made in the potting mixture of each container. A single plantlet should be placed upright in the hole and covered by gently pushing the potting mixture around the plantlet (Plates 4a, b).



*Plantlet removal from the flask*

Plate 2



*Washing of roots in water to remove agar or nutrient medium*

Plate 3



Plate 4a

*Plantlet transplantation into the potting mix: (a) in small plastic pots of 5 cm diameter; (b) in black polyethylene bags of 15 cm diameter*



Plate 4b

### **Step 5** Watering and misting

Plantlets need to be watered soon after transplantation. Although the soil mixture must be kept moist, there is a narrow line between too much and too little water. High relative humidity should be maintained by regular misting using a hand sprayer. Water and humidity are essential for plantlet survival because leaves and roots of tissue-cultured plants are poorly developed and unable to maintain the plantlets' water balance until several days after transfer to the potting mix.

Ideally, the relative humidity should remain above 90% during the initial development of the young plantlets. To achieve such a high relative humidity, transplanted plantlets are best kept in a specially made humidity chamber or box (see Step 6a). Although a short passage through a humidity chamber is beneficial to plantlet survival (there will be fewer losses of plantlets in the nursery), this step is not always essential or possible. As such, plantlets can also be kept directly in the main nursery area without special humidity treatment (Step 6b).

### Initial growth in a humidity chamber

If a humidity chamber is available, plantlets should have been transplanted in small plastic pots (5 cm diameter; see Step 2; Plate 4a). Pots should be placed in the humidity chamber (Plate 5) for about 1–2 weeks. New, small leaves and roots will have formed during this period. Plants should be kept out of direct sunlight at this stage (see Nursery Management, page 10). The plantlets should be regularly misted, depending on the nursery temperature. The presence of condensed water droplets on the inside of the sheet is indicative of sufficient humidity in the chamber. The sides of the plastic sheet should be slightly opened on the fifth day. A few days later, the sides should be completely opened and the sheet removed the following day. Watering should be continued at regular intervals.

### Step 6a



*Recently transplanted plantlets in a humidity chamber*

Plate 5

Various types of humidity chambers or boxes can be made easily using local materials. For example, a simple wooden frame 50 cm high can be covered with clear plastic sheeting and placed on the nursery floor.

### Step 6b Establishment in the nursery

Plantlets should be transplanted into black polyethylene bags (see Step 2), either directly after removal from the tissue-culture flasks (Plate 4b) or following 1–2 weeks growth in a humidity chamber (see Step 6a). Bags with plantlets are best arranged in double rows (Plate 6), which saves on space and still allows for proper leaf development on the side of the open alleys between rows. Bags or rows of bags may be labeled to indicate cultivar or genotype name and date of transplantation. Watering should preferably be daily, certainly during the first 2–3 weeks. Watering can be done using a water can, hosepipe, or sprinkler, depending on the facilities available in the nursery area. Care should be

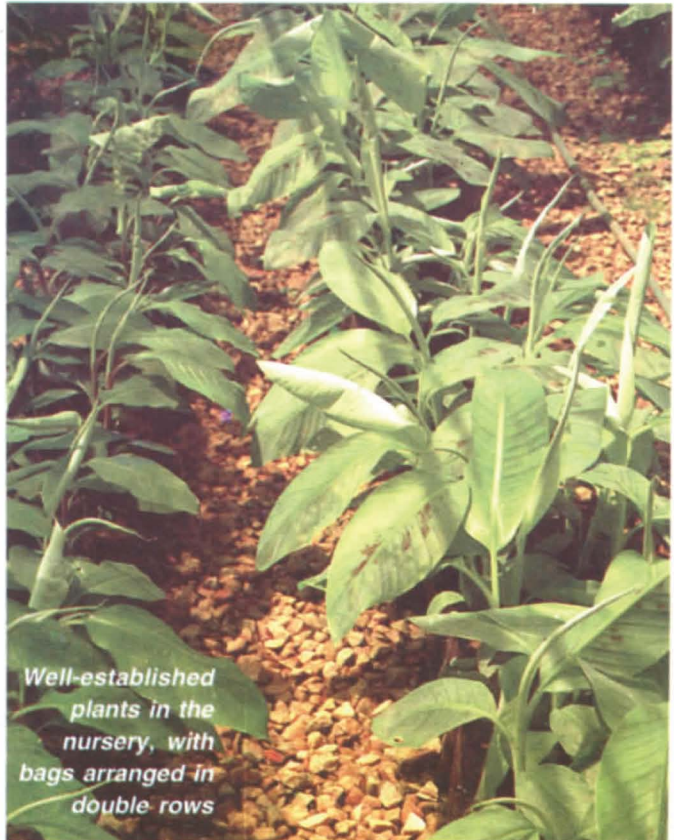


Plate 6

taken not to damage the potting mixture or plantlets by heavy splashing during watering. Plantlets should initially be kept in partial shade (50–60%), with a gradual increase in sunlight during the nursery stage (see Nursery Management, page 10).

Plants may be kept in the nursery for a period of 2–4 months before field planting. The length of the nursery period depends largely on temperature, in addition to potting mixture fertility and water and humidity. In hot, humid lowland areas (such as the humid forest zone of West and Central Africa), plants can be ready for field planting after only 2 months in the nursery. In cooler areas (such as the mid-altitude and highland zones of East Africa), plants may require up to 4 months to reach the required height for field planting (mainly due to cool nights). Plants should be 20–30 cm tall and have 3–5 broad leaves to be ready for field planting (Plate 7).



Plate 7

*Micropropagated banana  
plant for field planting*

## Nursery Management

### Nursery types

Various types of nurseries with different degrees of sophistication can be used to harden micropropagated banana/plantain plants. Simple sheds can be made from locally available materials, such as bamboo or other wooden sticks, roofed with mats of leaves or papyrus (Plate 8a). Stronger, permanent structures can be constructed from metal pipes in concrete slabs with chainlink and/or shadecloth as cover, and stone chippings on the floor (Plate 8b). Specialized greenhouses, screenhouses, or solar domes can be purchased from professional nursery suppliers, but these can be expensive.



Plate 8a



Plate 8b

*Nursery types: (a) simple shed made of wooden sticks and papyrus roof; (b) permanent nursery constructed from metal pipes with chainlink and shadecloth as cover, and stone chippings on the floor*

Plants will usually require daily watering because of the restricted root system. Watering is normally done once in the morning. When plants have developed several leaves and it is a dry season, watering may be required both in the morning and afternoon. Careful attention to watering is required at all stages. Too much water is as bad as not enough water.

Generally, air temperatures of between 15 and 35 °C, with an optimum of between 25 and 30 °C, should be maintained.

A high standard of hygiene is necessary to reduce the risk of damage by pest or pathogen attack. Caterpillars and mites can be serious leaf pests in the nursery. Spraying plants with insecticides has been effective for the control of such pests.

Partial shading is essential in the early stages of nursery establishment. Shade can be provided by using special shade cloth to cover the nursery or greenhouse frame or by roofing a simple shed with mats of papyrus, palm leaves, or other available plant material. Plants can be exposed steadily to greater sunlight by gradually reducing the shade until full sunlight just before field planting. This can be achieved by progressively removing the shade cloth or mats of leaves.

It is important that the mixture is free draining. In case only heavy soil is available, it should be mixed with sand to improve drainage. Chicken or cow manure can be mixed with the soil for fertility reasons. If this is not available, liquid fertilizer should be applied to avoid mineral deficiency problems. Young plantlets can easily be burned by fertilizer, so great care should be taken when applying it. Preferably, its effect should first be tested and monitored on a few plantlets over about 10 days. If a commercial liquid fertilizer is available, this could be used at quarter to half strength (that is quarter to half of the recommended concentration). Otherwise, a mixture of urea (0.1–0.5 g) and potash (KCl at 0.2–1 g), dissolved in 100 ml of water, can be applied per plant. Plantlets should be 3–4 weeks old before any fertilizer application is considered.

## Water

## Temperature

## Sanitation

## Light

## Potting mixture and fertilizer



## Field Planting and Management

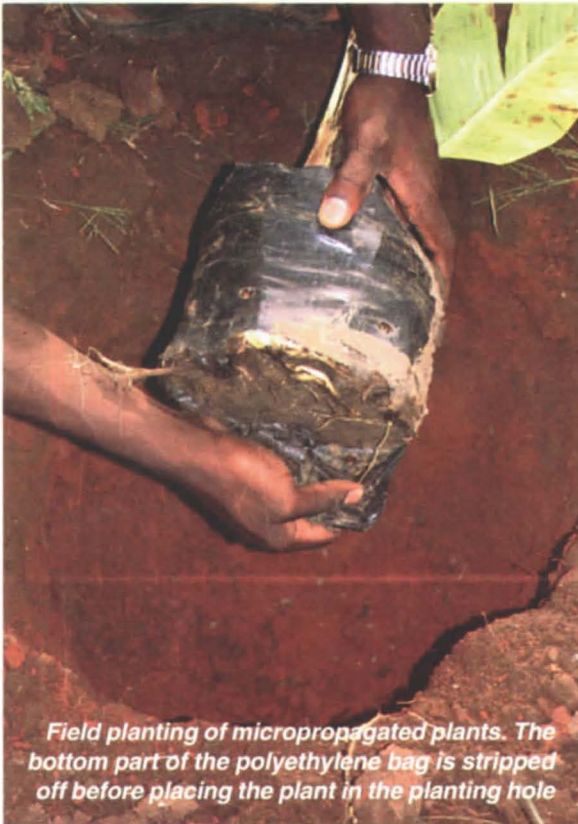
Plants should be kept in the nursery for 2 to 4 months until they are 20 to 30 cm tall (Plate 7). Such plants are the preferred material for field planting. However, if field planting must be delayed, e.g., because the planting time is not right or the rainy season has not yet arrived, plants can be kept for a longer period. If the delay will be significant, the space between plants can be increased by moving the nursery bags. Such wider spacing will prevent the plants from growing too long and slender, which would result in weaker plants. In case the delay in field planting becomes excessive and plants grow taller than 1 m, they can be cut back at 10–20 cm above pot level, 1–2 months before field planting.

Field planting should be done in the early morning or late afternoon to avoid heat. Plants should be watered well just before planting, transported to the prepared field, and placed next to the planting hole. To avoid damaging the roots during planting, the bottom part of the polyethylene bag should first be stripped off (Plate 9); the plant should then be placed in the planting hole, partly covered with soil to provide stability to the plant and its root-soil clump in the bag, followed by removal of the polyethylene bag (without its bottom) by gently pulling it over the leaves and the top of the plant. More soil should then be added to the planting hole according to recommended practices in the area. The first new leaves should be formed within 2–6 weeks after planting in the field (Plate 10).

Plants may be watered in the field if necessary and feasible. Young micropropagated plants cannot withstand dry weather conditions as well as conventional propagules from suckers or corms.

Special attention should be given to the plants during the first 3–4 months after field planting. In addition to adequate soil moisture, plants should be kept free of weeds (particularly grasses and creeping weeds) and mulched, manured, and fertilized according to recommended cultural practices in the area. For more information on field management of plantains in West Africa, refer to Swennen (1990). If tissue-cultured plants are planted in an area where goats or cows graze freely, plants should be fenced or otherwise protected, as the succulent leaves of young plants are a favorite food for livestock.

Plate 9



*Field planting of micropropagated plants. The bottom part of the polyethylene bag is stripped off before placing the plant in the planting hole*



*Field-established tissue-cultured plants form new leaves within 2-6 weeks after planting in the field*

Plate 10

If properly managed, tissue-cultured plants usually grow more vigorously and taller, and produce bigger and heavier bunches than conventional planting material. However, tissue-cultured plants of many banana and plantain cultivars often produce a certain percentage of off-types (plants that do not resemble the original cultivar). Most off-types are of poor quality and are not readily noticeable until after bunch emergence.

Because tissue-cultured plants are not known to many farmers, and because they require extra care and management for successful field establishment, it is generally recommended that research and extension personnel receive and handle such plants. Tissue-cultured plants are then best used to establish field nurseries for further conventional propagation of clean suckers, which can reliably be distributed to, and handled by banana and plantain farmers. Such a scheme is particularly useful when tissue-cultured plants are used for the introduction and dissemination of newly selected cultivars or hybrids.

## References and Further Reading

- Daniells, J., and M. Smith. 1991. Post-flask management of tissue-cultured bananas. ACIAR Technical Reports 18. Australian Centre for International Agricultural Research, Canberra, Australia. 8 pp.
- Diekmann, M., and C.A.J. Putter. (eds.) 1996. *Musa* spp. (2nd edition). FAO/IPGRI Technical Guidelines for the Safe Movement of Germplasm No. 15. Food and Agriculture Organization of the United Nations, Rome/International Plant Genetic Resources Institute, Rome, Italy. 26 pp.
- Gauhl, F., C., Pasberg-Gauhl, D. Vuylsteke, and R. Ortiz. 1993. Multilocational evaluation of black sigatoka resistance in banana and plantain. IITA Research Guide 47. International Institute of Tropical Agriculture, Ibadan, Nigeria. 59 pp.
- Swennen, R. 1990. Plantain cultivation under West African conditions. A reference manual. International Institute of Tropical Agriculture, Ibadan, Nigeria. 24 pp.
- Vuylsteke, D. 1989. Shoot-tip culture for the propagation, conservation, and exchange of *Musa* germplasm. Practical manuals for handling crop germplasm in vitro 2. International Board for Plant Genetic Resources, Rome, Italy. 56 pp.
- Vuylsteke, D., and R. Ortiz. 1996. Field performance of conventional vs. in vitro propagules of plantain (*Musa* spp., AAB group). HortScience 31: 862–865.
- Vuylsteke, D., R. Ortiz, S. Ferris, and J. Crouch. 1997. Plantain improvement. Pages 267–320 in Plant Breeding Reviews, vol. 14, edited by J. Janick. Wiley, New York, USA.

## About IITA

The International Institute of Tropical Agriculture (IITA) was founded in 1967 as an international agricultural research institute with a mandate for major food crops, and with ecological and regional responsibilities to develop sustainable production systems in tropical Africa. It became the first African link in the worldwide network of agricultural research centers supported by the Consultative Group on International Agricultural Research (CGIAR), formed in 1971.

IITA is governed by an international board of trustees and is staffed by approximately 80 scientists and other professionals from over 30 countries, and approximately 1,300 support staff. A large proportion of the staff are located at the Ibadan campus, while others are at stations in other parts of Nigeria, and in Benin, Cameroon, and Uganda. Others are located at work sites in Côte d'Ivoire, Ghana, Malawi, Mozambique, Tanzania, Zambia, and Zimbabwe. Funding for IITA comes from the CGIAR and bilaterally from national and private donor agencies.

IITA conducts research, training, and germplasm and information exchange activities in partnership with regional bodies and national programs in many parts of sub-Saharan Africa. The research agenda addresses crop improvement, plant health, and resource and crop management within a farming systems framework. Research focuses on smallholder cropping systems in the humid and subhumid tropics of Africa and on the following major food crops: cassava, maize, plantain and banana, yam, cowpea, and soybean.

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