



How to Establish a Tissue Culture Banana Hardening Nursery

2008

INTRODUCTION

Tissue culture (tc) techniques have been used in Kenya over the last ten years to produce large quantities of disease-free banana planting material. To ensure farmers' easy access to tc plantlets in various parts of the country hardening nurseries are critical. Hardening is a process that enables tc plantlets from the laboratory to gradually adapt to external environmental conditions. This brochure briefly presents the steps involved in the establishment of a tc banana hardening nursery.

THE TISSUE CULTURE BANANA PROCESS

The tissue culture banana process entails 4 main stages;

Stage one: Sourcing of starter material

This involves selection and obtaining suitable young suckers from the desired cultivar in a healthy vigorously growing banana orchard. The suckers are washed with tap water and soap to remove soil. They are then reduced in size before being taken to the laboratory where they are surface sterilized with a disinfectant. The clean plants are then cut leaving a small part of the corm which is placed into a growth media under sterile conditions. The plants (now referred to as explants) are then placed in a growth chamber under controlled temperature and humidity.

Stage two: Multiplication of explants

After about one month the explants start producing other small plants (referred to as plantlets). These are separated and placed into new media and consequently produce new plantlets. This multiplication process continues for a maximum of five cycles whereby each cycle multiplies at a factor of at least four shoots every month and about 1000 plantlets are produced in six months (Table 1).

Stage three: Rooting

The explants are then transferred to a medium which has a combination of hormones that makes them produce roots.

Fig 1 Stages of tc multiplication

I



Cleaning and division under sterile conditions

II



Multiplication in controlled and sterile conditions

II



II



Rooted plantlets ready for hardening



Table 1 An example of a multiplication schedule of *tc* banana plantlets

Month	1	2	3	4	5	6	7
Multiplication cycles	0	1/2	01	02	03	04	05
No. of plantlets	1	4	16	64	256	1024	4104

Stage four: Hardening Nursery

What is a tc banana hardening nursery?

This is a structure in which the plantlets from the laboratory are kept to enable them to adjust to the outside environment in preparation for field planting.

The nursery is important because;

- It enables careful nursing of the plantlets.
- The plantlets occupy little space and therefore save labour during their early unproductive life.
- The plantlets are protected from harsh field conditions in the early stages of growth e.g. unsterilized soil, weed seeds, and pests.
- Ensures uniformity in establishment and production.



Banana hardening nurseries under farmers' conditions

ESTABLISHING THE HARDENING NURSERY

Where should a hardening nursery be located?

- A nursery should be located in a banana growing area
- The nursery should be accessible
- Water should be available near the site
- The nursery should be free from pests such as insects, fungi, nematodes, weevils
- The nursery should be fenced to protect seedlings from both wild and domestic damage

Soil mixtures

The soil should comprise of top soil/virgin soil + any of the following gravel, rice husks, sawdust, wood shavings, wood bark, or sand at a ratio of 6 parts to 1 part. Fertilizer application is not necessary at this stage.

Soil sterilization

It is important to sterilize soil to kill harmful organisms such as soil-borne fungal diseases, nematodes and weeds. This can be done through either steam sterilization, soil solarization or chemical sterilization.

a) Steam sterilization procedure

This involves heating soil with a steam-air mixture. A simple steam sterilization kit is illustrated in figure 2. Two drums are required; the upright drum is filled with moist soil mixture. The horizontal one is half filled with water and then heated at 60-70⁰ C. The steam produced sterilizes the soil and then emerges through the top lid.

After the steam emerges from the top of the drum a thirty minutes (30) sterilization period is recommended. After which the heating can be stopped.

Steam sterilization improves the soil physical structure, it is harmless to beneficial organisms in the soil, fast, easy, cheap, more effective than chemicals, and user friendly. The main disadvantage is the use of firewood which may be costly and environmentally unfriendly.

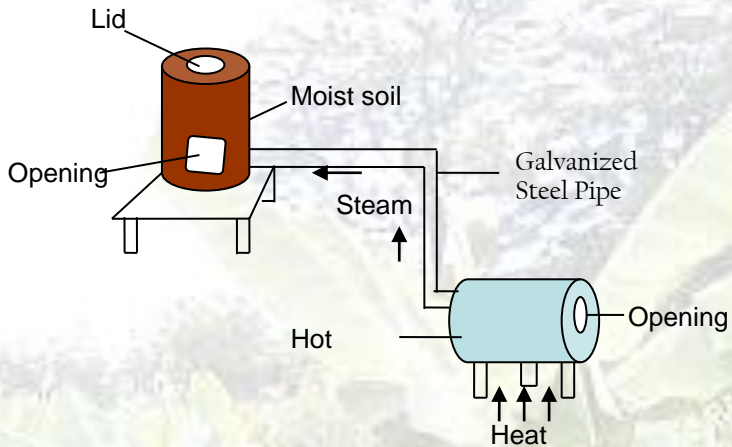


Fig. 1: A Simple Soil Steam Sterilization Kit

b) Soil Solarization

This involves using solar energy to heat the soil. The soil is collected and spread in a shallow 'pit' then covered with a clear polythene sheet in an open area to allow sunlight to penetrate. It takes 30-60 days depending on the weather.

b) Chemical Sterilization

This involves the use of chemicals. The commonly used chemicals are furadan (2kg per tonne of moist soil); mocap 10G (3kg per tonne) and Basamid (one tonne=2 pickup trucks =80 wheelbarrows). The soil is spread in a nursery-like bed, sprinkled with some water to moisten the soil. The chemical is mixed thoroughly with soil and the mixture covered with polythene. After one week the soil is turned over and covered again. After the second week the soil is completely uncovered turned and left open for any leftover fumes to diffuse out. The soil is ready for use after three weeks.

Note

Manure and compost should not be sterilized, but added after the soil has been treated.

Nursery structure

The materials required for the construction of a simple nursery shed and costs are given in Table 2. This cost can be reduced by utilizing locally available materials.

Nursery Tools and Equipment

Various tools are needed such as; potting bags, potting trays, polysheets, jembes, fork jembes, spades, rakes, trowels, buckets, wheel barrows, watering cans, horse pipes, and a store.

TRANSPLANTING OF PLANTLETS FROM LABORATORY TO NURSERY

Tunnels and/or trays into which the plantlets will be transplanted are prepared. The tunnels are prepared by making a nursery bed containing sterile soil mixture and covering it with a polythene sheet. Rooted plantlets are removed from the growth chamber. Trays are filled with sterile soil.

- Plantlets are carefully removed from jars, variety per variety.
- The media is washed off gently without injuring the plantlet.
- The plantlets are taken to the hardening nursery shed as soon as possible preferably the same day.
- They are transplanted into seedling trays or tunnels in rows and watered twice or once a day depending on weather conditions.
- One month later, they are transferred into 6 X 9 polythene bags containing sterilized soil and manure.
- Watering continues as necessary.

The seedlings are monitored regularly for somaclonal variants. Any plants showing unusual growth habits are removed and kept aside for further observation. Some varieties may show some purplish

coloration on the leaves which is normal in the early stages of growth. The seedlings are ready for field transplanting after two months when they are about one foot tall and have at least five leaves.

NURSERY HYGIENE AND ORGANIZATION

- The nursery should be kept clean and free from weeds
- Pests and diseases should be controlled immediately they are spotted.
- Seedlings should be arranged in neat rows with clear paths for walking and transportation.
- Varieties should be separated and labeled accordingly, showing information such as date of transplanting and number of seedlings for ease of marketing.

ADVANTAGES OF USING TISSUE CULTURE METHOD FOR MULTIPLICATION OF BANANA SEEDLINGS

- The planting material produced is free from pest and disease
- It is a rapid method of multiplication
- Uniformity in establishment and production is achieved
- Production of banana fruits is obtained after 9-10 months
- The yields are higher
- Easy transportation and /or importation of new varieties is made possible

DISADVANTAGES OF USING TISSUE CULTURE METHOD FOR MULTIPLICATION OF BANANA SEEDLINGS

- The price of tc plants is higher compared to conventional suckers.
- The plants require added care and management.
- The occurrence of off-types (somaclonal variants) is possible. These mutations are usually inferior and can lead to dwarfing or other undesired morphological features.
- Viruses can still be transmitted through tissue culture.

SOURCES OF SEEDLINGS

KARI-THIKA, JKUAT, and GITL

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SOME VARIETIES THAT ARE AVAILABLE INCLUDE;

**Grand Nain; Williams, Giant Cavendish, Dwarf Cavendish, Chinese
Cavendish, Chinese Dwarf, Lacatan, Paz, Valery, Pelipita, Solio,
Ng'ombe, Nusu Ng'ombe (Gradi) and Uganda Green.**

**COST OF ESTABLISHING A HARDENING NURSERY Capacity:
about 1500 plantlets**

Size: 4 M (Width) X 7 M (Length) X 3 M (Height)

ITEM	Price per Unit	Total (KSH)
Shade net 74 M 2	54.00/m2	3996
1 roll Chicken wire	5000	5000
8 Posts	160	1280
1 Door	2000	2000
Timber	-	4680
10 M Polythene sheet	100	1000
4 kg Nails	70	280
2 soil sterilization drums	2000	4000
1 Metal pipe	1000	1000
2 Elbows	50	100
1 union	100	100
1 ton Ballast	1000	1000
1 drum for water storage	2000	2000
1 hosepipe	1800	1800
2 watering can	150	300
1500 polybags	1	1500
Labour	5000	5000
Contingencies	10%	3455.6
Total		38,491.6

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