Technical Bulletin 5

GUIDE ON HANDLING AND HARDENING OF TISSUE CULTURED BANANAS

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Cover Photo

Front : An emerging bunch of tissue cultured banana

Back : Banana bract mosaic virus affected Plantain (Nendran)

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FOREWORD

Production of bananas and plantains in India is 15.07 million tonnes from an area of 4.64 lakh hectares, thus, emerging as number one in the world ahead of Brazil. This amounts to 17% of world production. The unique feature is that the entire Indian production is mainly contributed through small-farming system. It is targetted to achieve 25 million tonnes during the year 2020. Towards achieving such a challenging target, research and development programme are being strengthened and reoriented.

Banana production is constrained by several biotic and abiotic stresses of which the virus diseases assume greater importance. The other maladies caused by nematodes, bacterial and fungal pathogens are also serious. Many of the maladies are carried through suckers derived from mother plants, necessitating the need for healthy planting material.

Use of disease and pest free planting material is the basic need for successful production. Tissue cultured banana propagules have the potential to enhance productivity to a larger extent, if they are hardened properly. Since the recent past, tissue - cultured bananas are becoming increasingly popular, as they are vigorous and to start with disease-free. Tissue cultured banana plantlets, however, acquire several pathogenic bacteria, fungi and nematodes during secondary hardening. Extreme care, therefore, should be bestowed during hardening to get healthy propagules for reaping fully the production potential of tissue cultured bananas. This manual offers useful practical procedures for hardening of tissue cultured bananas beginning from net-pot stage to the final stage of field establishment and is useful to growers and technicians who are practically involved in the process of handling and hardening of tissue cultured bananas.

I hope it enables them to develop skilled green fingers.

Kalloo

Date : 14 Dec, 2001

(G. Kalloo)

Deputy Director General (Hort.)
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GUIDE ON HANDLING AND HARDENING OF TISSUE - CULTURED BANANAS

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INTRODUCTION

Tissue culture as a method of mass multiplication of bananas and plantains becoming more popular among researchers and growers. The advantages of tissue culture, in addition to faster rate of multiplication, include production of clean or disease free planting material and the small amount of space required to multiply large number of plants. Tissue culture has greater role in the safe movement of germplasm and planting material between the countries. It offers the mechanism of international dissemination and propagation of newly selected and developed cultivars or hybrids of banana and plantain. In the context of the ravages by the dreaded Bunchy top virus disease of the hill banana Virupakshi (Pome-AAB) in the hills of Dindigul District of Tamil Nadu it becomes necessary to produce and supply Bunchy top virus - free planting materials for better revival of hill banana plantations.

Tissue culture plants are produced in a closed, sterile environment and grown in a nutrient rich artificial medium under controlled conditions. When removed from the controlled environment, the plant requires to adjust to the outside environment which has varying light levels, changing temperature, reduced humidity, low nutrient availability and pressures from pathogens. This acclimatization is achieved during hardening in the nursery specially designed.

This manual provides a stage by stage practical procedures for successfully handling tissue culture bananas from net pots to the final stage of field establishment. The authors have drawn conclusions from their own practical experiences and scientific publications available.

HANDLING

If tissue cultured plants are obtained in net pots, proper handling of such flask is vital for successful establishment outside the laboratory.

The transit time should be minimum and properly prepared and packaged to withstand transit stress. Prolonged periods of darkness, such as in CFB boxes used for transport, may cause weakening of plantlets and reduced survival rate. Temperatures below 10^0 C and above 40^0 C should be avoided. The packages should be protected from direct sunlight and be kept in an upright position. The transit time exceeds 48 hours, it is desirable to occasionally expose the flasks to light, if possible.

Tissue cultured plantlets be taken to the nursery as soon as possible upon arrival. If this is not possible, remove the plantlets in net pot from the package and keep them in a relatively clean room with indirect sunlight or fluorescent light. A room temperature of 20^0 - 35^0 C is adequate. Tissue cultured plantlets which are ready for transfer to polybag, can usually be kept in the net pot for another 2-3 weeks.

Microbial contaminations should be monitored up to 2 weeks and infected plantlets should be destroyed. For best results the plantlets should measure 7-10 cm tall with strong root system (plate 1,2 and 3) Most of the commercial tissue culture laboratories supply primary hardened plants in net pots which are cheaper.

SECONDARY HARDENING

Potting mixtures

Good quality pot mixture is essential for successful establishment and growth of plantlets. Different potting mixtures can be used for hardening

a) Peat soil + well decomposed and powdered farm yard manure (FYM) in the ratio of 12:1.

b) Sand + top soil + vermicompost in the ratio of 3:1:1.

c) Sand + FYM + vermicompost + red soil in the ratio of 2:1:1:1

Sieve the soil and sand to eliminate stones, stubbles and gravels before preparing the mixture. Use of vermicompost substituting FYM may prevent the incidence of bacterial wilt which is common in young tissue cultured plantlets. Addition of neem oil cake at the rate of 50 grams per kg of potting mixture can help reducing nematode incidence during hardening.

1. A view of Tissue Culture plants in net pots

2. A closeup view of Tissue culture plants - primary hardening

3. A tissue culture plant in netpot

4. Removal of plant from netpot for transfer

5. Transplanting in polybag

6. Secondary hardening in nethouse

7. Erwinia soft rot

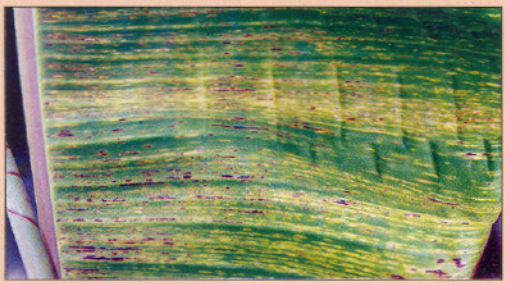
8. Bacterial rot

9. Infestation of rootknot nematode

10. Tissue culture plant ready for field planting

Viral diseases of Banana

Banana Bunchy top



Banana Streak

Banana Mosaic (CMV)

Banana Bract Mosaic

7. Erwinia soft rot

8. Bacterial rot

9. Infestation of rootknot nematode

10. Tissue culture plant ready for field planting

Bag Filling

The potting mixture has to be filled to the brim in black polyethylene bags (150 gauge) of the size 15 cm diameter and 30 cm height. If bag size is reduced, the time taken for secondary hardening would be more than 45 days.

Plantlet Transplantation

Plantlet transplantation in bags should not be in the direct sunlight and it should be done in partial shade. Plantlets should be removed from the net pots very carefully without much damage to the tender roots. Using a scissor or knife the net pot can be cut and removed just before planting (Plate 4).

The bags should be watered a day prior to transplanting. A small hole of about 5 cm deep can be made. Plant the plantlets into the hole in the upright position and cover with potting mixture and press gently around the plantlet (Plate 5). Irrigate the transplanted plants on the same day. The next day drench the bag with 100 ml of Emison - 6 at 0.1% to guard against fungal and bacterial contamination. Also add 3 grams of Carbofuran 3G to prevent nematode infestation.

Water and misting

Plantlets need to be watered to keep the mixture adequately moist. Although the soil mixture must be kept moist, there is a narrow line between too much and too little water. For maintaining good growth, high relative humidity and partial shading are necessary as the leaves and roots of tissue cultured plants are poorly developed and unable to maintain plantlets' water balance until several days after transplanting. Use of 75% shadenet and regular misting of water can create necessary environment for growth and hardening. Idealy the relative humidity should remain above 90% for atleast a week.

When plants have developed several leaves, irrigate morning and evening, if it is a dry season and once in the morning in cool season.

ESTABLISHMENT IN THE NURSERY

The polythene bags with plantlets are best arranged in double rows (plate 6) which saves on space and still allows for proper leaf

development on either side of the open alleys between rows. Watering should preferable be daily, certainly during the first two to three weeks. Use of low head sprinkler can save cost of labour. The plants may be kept in nursery 40-60 days before field planting. The length of the nursery period depends largely on shade, temperature, size of bag, potting mixture fertility, moisture and humidity. The light intensity can be regulated by using shade nets. It is recommended to use 75 per cent shade net. A week prior to planting in the mainfield, shade nets can be removed to expose the plants to full sunlight.

Plants would be ready for field planting when they attained a height of 20-30 cm tall with 3-5 broad leaves (Plate 10).

Spreading coarse sand in the hardening nursery is useful to regulate temperature and humidity. Keeping the plants in insect proof net house can help ward off insect vectors transmitting banana viruses. This is essential in areas where virus diseases are prevalent.

Temperature

Generally a temperature of between 15^0 C and 35^0 C, with an optimum of between 25^0 C and 30^0 C, should be maintained.

Manuring

Plantlets should be 2-3 weeks old before any fertilizers application is considered. Liquid fertilizers should be applied to avoid mineral deficiency. A mixture of urea 0.5 g and muriate of potash 1 g dissolved in 100 ml of water can be applied per plant. Add 2 g of superphosphate mixed in water for better root development. Repeat the schedule by doubling the dosage after 3 weeks. Spraying the plantlets during sixth week with commercially available micronutrient mixture would result in better establishment in nursery as well as in field.

PLANT PROTECTION

A high standard of hygiene is essential to minimize the risk of damage by pests and diseases.

The following pests and diseases are often encountered during hardening process.

Bacterial soft rot *(Erwinia carotovora)*

Young roots of tissue cultured plants are often invaded by *Erwinia* bacterium causing soft rotting of corm. The affected plants do not produce new leaves, remain stunted and present dehydrated appearance. The core leaf (central leaf) either remains unopened or partially opened and gradually becomes yellow, later turning to brown. The symptom will spread from central leaf to adjacent leaves gradually. The roots are poorly developed and often become black. Cross section of corm may exhibit yellowish brown soft rotting extending up to the growing point (Plate 7 & 8). Occasionally the bacterium may attack the neck region and the leaf symptoms will appear from the peripheral leaves towards the centre.

To control the disease, the soil in the bag should be drenched at transplanting, two weeks later and 10 days prior to transplanting in the mainfield with 50 ml of 1 per cent mercuric chloride or 50 ml of 0.1 per cent Emison - 6. Spraying streptocyclin sulphate to 3 weeks old plantlets minimizes bacterial soft rot considerably.

Leaf spot diseases

Many fungal leaf spots may occur as the nursery environment is congenial for the growth of the organism. One or two sprays with 0.1 per cent carbendazim or 2.5 per cent copper oxychloride is essential. The number of spray cycles depends on the severity of incidence. Always add wetting agent @ 5ml / 10 litre of water.

Nematodes

The tissue cultured plantlets are more susceptible to nematodes like root knot nematode (Plate 9), lesion nematode, burrowing nematode and spiral nematode as the roots are very tender. It is very essential to take up nematicidal application from the beginning. Apply 3g of carbofuran 3G at planting in the bag, 4th week and a week before taking to field planting. **Failure to do so would result in heavy incidence and multiplication of nematodes in the mainfield resulting in considerable casuality.** Application of one gram in each of Azospyrillum and phosphobacterium and 10 g of vesicular arbuscular mycorrhizae (VAM) per plant would be benefical for better root development besides reducing the nematode population.

FIELD PLANTING AND INITIAL MANAGEMENT

Plants should be kept in the nursery 40-60 days until they are 20 to 30 cm tall with 3-5 broad leaves. Weaker plants should be separated and kept separately for further maintenance until they attain plantable stage. If field planting must be delayed plants can be kept for a longer period. In such cases, the space between the plants can be increased. Such wider spacing will prevent the plants from growing too tall and slender, which would result in weaker plants. In case the delay in field becomes excessive and plants grow taller than one metre, they can be cut back at 10-20 cm above bag level 1-2 months before planting. Field planting should be done early or late afternoon to avoid heat.

Plant should be watered well. Just before planting bags are transported to prepared field and placed by the side of planting pit. To avoid damage to roots while planting, bottom part of the polythene bag should be cut and stripped off, then the bag should 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 polythene bag (with out its bottom) gently pulling it over the leaves on the top of the plant. More soil should then be added to the planting pit according to the recommended practices in the area. The first new leaves should be formed within 2-4 weeks of planting in the field.

Plants should be watered in the field soon after planting as young micropropagated plants cannot withstand dry weather and heat. **While planting in the field, apply 10g of carbofuran 3G per plant. Within 3 days of planting, drench the soil around the plants with 500ml of 0.5 per cent Emison - 6 to guard the plants against bacterial soft rot to which the tissue cultured plantlets are highly prone to.**

It is necessary to reiterate that the tissue cultured plants are only pests and disease free at the time of planting. In the early stage of transplanting they are more prone to pests and diseases. It is, therefore, very important to adopt suitable plant protection measures prophylatically, for better growth and development later.