Technical Bulletin No. 3

MULTIPLICATION OF VIRUS FREE BANANA PLANTS THROUGH SHOOT TIP CULTURE

S. Sathiamoorthy
S. Uma
R. Selvarajan
B. Shyam
H.P. Singh

भाकृअनुप
ICAR

NATIONAL RESEARCH CENTRE FOR BANANA
(Indian Council of Agricultural Research)
17 Ramalinganagar south extension, Vayalur Road,
Truchirappalli - 620 17, Tamil Nadu, India.

MULTIPLICATION OF VIRUS FREE BANANA PLANTS THROUGH SHOOT TIP CULTURE

S. Sathiamoorthy
S. Uma
R. Selvarajan
B. Shyam
H.P. Singh

NATIONAL RESEARCH CENTRE FOR BANANA
(Indian Council of Agricultural Research)
17 Ramalinganagar south extension, Vayalur Road,
Tiruchirapalli - 620 17, Tamil Nadu, INDIA.

Technical Bulletin No. 3

Multiplication of Virus Free Banana Plants Through Shoot Tip Culture

Editors

S. Sathiamoorthy, S. Uma, R. Selvarajan,
B. Shyam and H.P. Singh

Citation

S. Sathiamoorthy, S. Uma, R. Selvarajan, B. Shyam and H.P. Singh (2001)
Multiplication of Virus Free Banana Plants Through Shoot Tip Culture.
National Research Centre for Banana (ICAR), Tiruchirapalli, India.

Photography

S. Sathiamoorthy

Cover Photos

Front : Tissue culture banana plant with bunch
Inset : A ready to plant tissue culture banana in poly bag.

Back : Bract mosaic virus infected Red Banana

Published by

Director,
National Research Centre for Banana (ICAR),
17, Ramalinganagar South Extension,
Vayalur Road, Tiruchirapalli - 620 017,
Tamil Nadu, INDIA.

Layout & Printed at

Niseema Printers, S. Kalamassery, Kochi, Kerala, India. Ph : 0484 - 559708.

Foreword

Indian fruit industry is dominated by banana and plantain with an annual production of 13 million tonnes from an area of 4 lakh ha. This spectacular increase in production and productivity over the last two decades is mainly due to the great improvement in production and post harvest handling technologies which include extensive adoption of high yielding varieties, high density planting, paired row planting system and drip irrigation.

Of late, banana production is threatened by many biotic factors of which the viral diseases pose a major concern. The ambitious vision of 25 million tonnes of banana production by the year 2020 seems to be hampered by the alarming spread of viral diseases like Banana Streak Virus (BSV), Banana Bract Mosaic Virus (BBMV), Banana Bunchy Top Virus (BBTV) and Cucumber Mosaic Virus (CMV).

This only implies that access to technology information on the nature of diseases, control measures is vital. In this context, the National Research Center for Banana at Thiruchirapalli has brought out this bulletin which encompasses the details, the steps for the production of clean planting material with special emphasis on viral diseases of banana. The symptomatology of different viruses given with illustration, would be very useful to identify the infected mother plants. Problems encountered during hardening and integrated scientific and practical methods to overcome the problems are well discussed.

I am sure that this publication will be of immense use to all those involved in banana production and research, starting from the grass root level farmers to private entrepreneurs involved in mass multiplication through tissue culture. This bulletin will pave way to get rid of virus affected planting material at field level for targeted production and productivity of banana through sustained research and developmental efforts. I congratulate the authors for bringing out this publication for the benefit of all those involved in banana research, extension and production.

Tamil Nadu Agricultural University
Coimbatore-641 003. T.N.

(Prof. Dr.S.KANNAIYAN)
Vice-Chancellor

21st February, 2001

Preface

Banana is the second largest fruit crop in the world trade with an annual production of about 88 million tonnes. With high nutritive values, banana and plantains are regarded as the food-fruit crop grown extensively for subsistence cultivation. India contributes to 15% of the total global production with 13.5 million tonnes from 4.00 lakh hectares. It has a great socio-economic significance. It is being grown widely in different agro-climatic conditions.

In the visionary approach for the year 2020, India has the target of 25 million tonnes which needs to be met out of dwindling natural resources and against pressures from various biotic stresses. Among the various constraints, viral diseases are posing a serious threat. Banana Bunchy Top has totally annihilated hill bananas grown in about 18,000 hectares in Tamil Nadu state. Since the recent past, new virus diseases such as Bract Mosaic Virus, Streak Virus and Infectious Chlorosis are causing further crop loss.

All these virus diseases spread rapidly from place to place through the use of infected planting materials. Realisation of the fact that clean and disease free planting is the foundation for the success of banana growing, technologies have been developed for mass multiplication through tissue culture. Use of conventional suckers does not offer any guarantee against freeness from viral infection. Use of tissue culture plants would greatly relieve the problem of obtaining good propagules. In this process, selection of good starting material is very vital.

In this bulletin, efforts are made to describe criteria for mother plant selection and virus indexing techniques at various stages. The hardening techniques described would be useful to the growers to take-up secondary hardening of net-pot plants by themselves to reduce the cost of planting material.

We hope that the bulletin would be of immense use to entrepreneurs, researchers, students and growers. The illustration and descriptions of virus diseases would help identifying the diseases and eliminate them in the field and mother blocks intended for collecting explants

We thank Mrs. Dayarani, Research Associate, G.Rajagopal, Senior Research Fellow and P.Druai, T-3 for their sincere assistance in bringing out this bulletin.

AUTHORS

CONTENTS

MULTIPLICATION OF VIRUS FREE BANANA PLANTS THROUGH SHOOT TIP CULTURE

INTRODUCTION

Globally, banana is fourth important food crop in terms of gross value exceeded only by paddy, wheat and milk products and forms an important crop for subsistence farmer as security for food and income. It is grown in over 4.0 lakh hectare with an annual production of 13.2 million tonnes. There has been an unprecedented increase in production and productivity in the country owing to adoption of improved production technology. Yet, to realise the potential yield for targeted production, constraints limiting the production have to be alleviated. Biotechnological approaches have facilitated in ameliorating many of the problems in banana production and improvement.

Traditionally, banana is being propagated using suckers or corms, but realizing the advantages of *in-vitro* propagation, mass multiplication through shoot tip culture has been gaining popularity world over as a novel approach for the production of disease free plants. First successful *in vitro* clonal propagation of banana was reported in 1960's. Since then *in vitro* culture of banana has undergone a lot of refinements and has become a commercial venture owing to its many advantages. Planting material with inherent high multiplication rate per unit time, better establishment with hardening, uniformity at flowering and harvest, higher returns with increased yields and freeness from nematode, fungal and bacterial pathogens are some of the advantages which attracted commercial firms in India for mass multiplication using tissue culture techniques. However, its performance has been varying and success of shoot tip cultured plants has become debatable which is attributed to lack of systematic phytosanitation resulting in appearance of viruses like Banana Bunch Top Virus (BBTV), Banana Streak Virus (BSV) and Banana Bract Mosaic Virus (BBMV) in *in vitro* propagated plants.

PRODUCTION OF DISEASE FREE PLANTS

Shoot tip culture forms the basis of production of disease free plants. As it is well packed with leaf primordia, possibility of entry of fungal, bacterial, nematode and insect pest is negligible or rare. But with respect to virus, it is assumed that fast growing meristematic cells outgrow the virus multiplication and are devoid of viruses. The cell to

cell movement of virus is also much slower than the meristematic growth and hence meristem culture increases the possibilities of virus elimination. Success of *in vitro* propagation, however will depend upon selection of proper mother plants, insurance for freeness from viruses and elimination of viruses. The following are the steps for successful mass propagation through shoot-tip.

* Identification of mother plants and initial nursery screening
* Virus indexing.
* Aseptic meristem tip culture initiation of indexed plants.
* Multiplication of propagules.
* Hardening of plants.

IDENTIFICATION OF MOTHER PLANT

Selection of a mother plant is very vital for *in vitro* propagation to have maximum benefit. Utmost care is required to be taken to select the mother plants which are true to type, have high yield potential, apart from its freeness from viruses. The following points should be borne in mind while selecting the mother plants.

* Plant should be healthy as evidenced by normal growth pattern.
* Pseudostem should be sturdy to support the bunch
* Plant should have 13-15 photosynthetically functional leaves at any vegetative stage and 5-6 leaves at harvest.
* Plant should have a shorter crop cycle.
* Plant selected should have higher bunch mass compared to the population mean with pendulous hang.
* Fruit quality should be representative of cultivar concerned.

DESCRIPTION OF COMMERCIAL CULTIVARS

India has an array of cultivars grown throughout the country depending upon regional preference, resource availability and production system. Dwarf Cavendish and Robusta are widely adopted commercial cultivars owing to high yield, wide market acceptability, ability to withstand wind, short cropping duration and high economic returns

per unit area. But recently some exotic cultivars like Grand Naine, Williams etc. have proven superior in farmers field owing to their sturdy stature and high yield potential. Poovan is another important cultivar grown widely due to wide range of adaptability to marginal conditions and tolerance to many biotic and abiotic stresses. Rasthali is significant in commercial production especially for its excellent quality of fruit. Nendran (Plantain) is an important cultivar grown in Kerala and Tamil Nadu. The important cultivars and selection criteria within sub-group are described.

Cavendish clones (AAA)

Dwarf Cavendish

Synonyms : *Basrai, Bhusawal, Jahaji, Kabuli, Pacha vazhai, Mauritius, Morris, Kuzhi vazhai, Sindhurni and Singapuri*

It is the leading commercial cultivar contributing to 58 per cent of total production owing to its high yield, ability to withstand strong winds, short crop cycles, good responsiveness to micro-irrigation and high economic returns per unit area. Its cultivation is concentrated more in Maharashtra, Madhya Pradesh, Gujarat and Bihar. Stature is short 1.5-2.0 m stout, yellowish green and covered with unevenly spread dark brown-black blotches. It possesses persistent male flowers. Ripe fruits are pale yellowish green in colour in tropics, while in cooler climate it is yellow exhibiting brownish dots spread all over the peel at maximum ripening. Several superior Cavandish clones have been identified and are under advance stage of evaluation. A selection known as 'Hanuman' or 'Padarse' is gaining popularity despite longer crop duration. This selection produces bunch weighing 55-60 kg. and performs better under light soil condition with higher inputs.

Robusta

Synonyms: *Bombay green, Pedda Pacha Arati, Harichal, Bor Jahaji*

It is a semi-tall sport of Dwarf Cavendish. It is an important cultivar in Karnataka, Andhra Pradesh and parts of Maharashtra. It bears a good bunch weighing 25-30 kg with good sized slightly curved fruits. Pseudostem is 1.8-2.5 m. tall, yellowish green in colour with conspicuous brown black blotches. Plant takes approximately an year to complete its life cycle and is annually planted. Propping requirement makes the crop investment-intensive. Its high susceptibility to Sigatoka diseases limits its cultivation in humid areas and is resistant to Panama disease race 1 and race 2.

Grand Naine

It is a selection from Giant Cavendish, identified to be major export variety introduced in India in the recent past. Plant characters resemble Cavendish for most of the parameters except for its robust stature, well spaced hands with straight fingers of bigger size. It bears heavy bunch weighing 25-30 kg and requires propping.

Silk (AAB)

Synonyms: *Rasthali, Amirthpani, Malbhog, Mortman, Rasabale and Poovan (Kerala)*

This is the choicest table variety for its tasty, crisp, good sour-sweet blended and pleasant apple flavoured fruits. Plant is medium statured with yellowish green stem and pink pigmentation on petiolar blades. Crop takes about 13-15 months to come to harvest with bunch weight of 15-18 kg. Bunch hangs at 30° angle from pseudostem. The male flowers have distinguishing pink dotted stylarneck. It has about 6-7 hands with bold, stout fruits turning golden yellow on ripening. Hard lumps and fruit cracking are the major physiological disorders, while *Fusarium* wilt race 1 and leaf spot diseases are its major production constraints.

Mysore (AAB)

Synonyms: *Poovan, Alpon, Champa, Chini Champa, Dora Vazhai, Karpura Chakkarakeli, Mysore, Palayankodan etc.*

Poovan is the cultivar grown all over the country in a perennial cropping system of cultivation. It is the leading commercial cultivar of southern and north-eastern states. It is distinguished from other cultivars by its pink pigmentation on the ventral side of the midrib. It bears heavy bunches as high as 24 kg with closely packed short, bottle necked fruits having a conspicuous nipple. Number of fruits per bunch varies from 150-300. Though fruits are slightly acidic and crop duration is 12-13 months, its ease of cultivation and hardness makes Poovan a popular cultivar. However, it is highly susceptible to Banana Streak Virus (BSV) and most recently Banana Bract Mosaic Virus (BBMV).

Plantain (AAB)

Synonyms: *Nendran, French plantain*

Nendran is believed to have originated in southern India from where it had spread

to Africa and diversified. It is the most prized cooking variety used in Kerala, fetching premium price during festive occasions. The banana products exported till date of Gulf countries are only of Nendran. Cultivation of this cultivar is also spread to parts of Tamil Nadu. Plant is tall and slender with distinct pigmentation in younger leaves and pink shade on the pseudostem. Male axis is not naked but is covered with persistent male bracts and flowers. Bunch weight varies from 10-15 kg having 30-50 fingers. Fruits have a distinct neck with thick green skin turning buff yellow on ripening, fruits remain starchy even on ripening. It is highly susceptible to Banana Bunchy Top Virus (BBTV), Banana Bract Mosaic Virus (BBMV). It is also susceptible to Neer Vazhai, malady of unknown etiology where fingers remain ill filled and remain only pencil thick. A number of ecotypes of Nendran namely, Zanzibar, Otta Moongil, Moongil, Kali Ethan, Vali Ethan, Manjeri Nendran, Chengalikodan, Nedu Nendran, Chenganacheri Nendran, Attu Nendran, Myndoli, Padali Murian are known and to have perhaps originated as bud mutants. Nendran is more susceptible to bract mosaic virus and leaf diseases like Black Sigatoka.

Unique clones

Red banana (AAA)

Synonyms: *Lal Kela, Chenkadali, Sevvazhai, Yerra arati, Chandra bale, Kembale*

This is an elite variety grown for its attractive red skin and delicious fruits. Pseudostem is reddish, robust and 2.5-3.0 m tall. Bunch orientation is 45° from the pseudostem. The male axis is also pigmented, hands are followed by a bare fertile axis. It yields bunches weighing 20-25 kg having 70-90 fruits. It has long crop duration of 16 months. Its commercial cultivation is limited to specific regions. It is also a popular backyard clone. It is highly susceptible to leaf diseases, Fusarium wilt, nematodes and the viruses like BBTV and BBMV. Although this cultivar was grown only in backyard garden, of late, large scale cultivation is being practiced due to increasing demand for fruits in Kerala and Tamil Nadu.

Monthan (ABB)

Synonyms: *Bontha, Karibale, Bainsa, Kalyan Bale, Ounda Bale.*

It is a fairly tall and robust plant growing to a height of 2.5-3.0 m. Stem is yellowish green without pigmentation and very shiny. Inflorescence is bold and hangs parallel to the pseudostem. For the reason that male flower and pseudostem pith are highly rel-

ished as vegetables and are of heavy yielding capacity, this culinary variety, till now restricted to backyards, has entered commercial cultivation in states like Tamil Nadu, Kerala and Andhra Pradesh and Orissa. Monthan bears a bunch weighing 20-25 kg. with 60-70 fruits which are bold, stocky, knobbed and pale green in colour. Apart from culinary purposes, few allied members of Monthan are suited for making chips. It is susceptible to Fusarium wilt race-4 and leaf spot diseases.

Ney Poovan (AB)

Synonyms: *Njali Poovan, Elakki Bale, Ney Kadali, Hoobale, Vadakkan Kadalai, Deva Bale, Putta Sugantha, Safed Velchi, Chini Champa.*

Ney Poovan is an unique diploid assuming commercial monoclonal cultivation on a large scale. Once, a delicate backyard cultivar of choice, Elakki Bale has occupied large areas in Karnataka under cultivation. It is a slender, medium tall plant taking about 12-13 months for its crop cycle. The bunch orientation is horizontal (18-20 kg wt) with small fruits packed closely having a wind blown appearance. Pulp is ivory coloured, firm, sweet, having good aroma with conspicuous ovules. Fruits have good keeping quality. It fetches double the price of other cultivars. Highly susceptible to BBMV and Fusarium wilt.

Pisang Awak (ABB)

Synonyms: *Karpooravalli, Kanthali, Jammulapalem collection, Pisang Awak, Pey Kunnan*

It is a hardy crop getting popularity in marginal soils. Drought, salt and wind tolerance, ease of cultivation and high productivity have favoured its commercial cultivation in Tamil Nadu and other states. Plant is tall 3.0-3.1 m in height, robust with light pink streaked pseudostem. Takes long period to come to harvest (14-15 months) but bears bunches weighing 25-35 kg. Fruits are neatly arranged with a spring like geometric orientation. Very sweet fruits are conspicuously ash coated, beaked and do not drop off when ripe making it suitable for long distance transportation. With its suitability for candy, juice and wine preparation, this variety has a better future.

FHIA-01 (Gold Finger)

The exotic hybrid belongs to Pome group with genomic constitution of AAAB. This hybrid has become commercial in Australia and in evaluation trials conducted at

NRCB, it has proved its potentiality for commercial adoption. This is resistant to Sigatoka and Fusarium wilt and produces bunch weighing 18-20 kg. Fruit quality is comparable with Pachanadan. Thus, this hybrid appears to be the best alternative to Pachanadan (AAB-Pome) which is highly susceptible to wilt race -1.

SELECTION CRITERIA OF DIFFERENT CULTIVARS

Cavendish clones-Plant should complete its life cycle within 12 months. Leaf orientation is preferably towards erect to facilitate high density planting. Yield should be consistently more than 35 kg. Bunch orientation must be pendulous. Fruits should have uniform upward orientation.

Silk clones-Plant should be sturdy to withstand strong winds. Plant should complete the life cycle within 12-13 months with a yield should exceeding 22 kg. Fruit orientation on the bunch must be pointing upward which would facilitate bunch transportation without damage. Fruits should exhibit thick skin (0.5-0.7 mm) to overcome fruit cracking. Fruits should turn golden yellow upon ripening and pulp should remain mealy without hard lumps.

Mysore clones-Plants with robust pseudostem must be selected. Plant should complete its life cycle within 12-13 months. Plant with cylindrical bunch shape is preferred over plants with a conical bunch. Bunch should exhibit symmetrical arrangement of hands and upturned fruit orientation to facilitate compact packing. The yield should be more than 22 kg under optimum conditions.

Plantain clones-For French Plantain (Nendran), the plant stature should be robust with a crop duration not exceeding 11 months. The bunch weight should be not less than 15 kg with fruits drooping down slightly at full maturity. The fruit pulp should have deep orange yellow colour. For Myndoli (Giant Plantain) yield should be not less than 40 kg.

Red Banana-Plant with a sturdy pseudostem, measuring 2.5-3.0 m tall is preferred. Pendulous bunch with a slight angle is desirable. Thick skinned fruits resistant to cracking with deep yellow pulp, pulp:skin ratio not less than 3.5 are desirable. Skin thickness should range between 3.0-3.5 mm. Crop duration should be 13 months in coastal regions and 15 months in subtropics.

Monthan-Robust plants with 13-15 photosynthetically active leaves at any stage

of plant growth. Plant should be a heavy yielder with more than 25 kg in case of Monthan subgroup and 30 kg with Bluggoe. Fruits should exhibit longer green life of 8 days at maturity, Pulp to peel ratio should be more than 2.5

Ney Poovan-Plant should be short (2.5 - 2.75 m) and sturdy to withstand wind damage. Plant should complete its life cycle within 12 months. Plants with angular bunch orientation should be preferred over horizontal ones. Comparatively thick skinned fruits are preferred to overcome the physiological disorder of fruit cracking.

Pisang Awak-Though Karpuravalli is tolerant to most of the pests and diseases, it is highly susceptible to Fusarium wilt. Hence care is to be taken to select a plant from wilt free plot. Mother plant should be robust, medium tall (3.0-3.5 m) and completing its crop in 14 months. Bunch yield should be more than 30 kg and should mature in 80-85 days. Its ecotype Kanthali is a better substitute to local Karpuravalli which is similar in all respects except for short stature.

VIRAL DISEASES OF BANANA

The mother plant selected has to be ensured virus free by symptom observation as well as through sero-diagnostic methods. Though most of the viruses have characteristics symptoms, they vary with variety, age of the plant and prevailing weather conditions. Many a time the symptoms are latest and are not expressed due to various crop growth conditions. Hence it is important to select plants free from inherent viruses though they are externally symptom free. Banana, the vegetatively propagated tropical fruit crop is infected by four viruses viz., Banana Bunchy Top Virus (BBTV), Banana Mosaic/infectious chlorosis caused by cucumber mosaic virus, Banana Bract Mosaic Virus (BBMV), and Banana Streak Virus (BSV). All the four viruses have been reported from India. In recent times, banana is commercially propagated through tissue culture. Survey report of NRCB reveals that the tissue cultured commercial varieties are found to be infected with viruses. Important viruses infecting banana, characteristic symptoms, particle morphology and transmission and indexing techniques are described.

Banana Bunchy Top (BBTV)

Symptoms

The presence of interrupted dark green streaks along the secondary veins of lamina or along midrib or petiole is considered to be the definite and reliable symptom of BBTV

infection. Leaves become progressively shorter, develop marginal necrosis and are also upright or bunched at the apex of the plant. If the infection is late, leaf symptoms do not appear and the bunch size is small and some times results in bunch choking.

Susceptible cultivars

All commercial cultivars are found to be susceptible. Robusta, Grand Naine, Virupakshi, Poovan, Sirumalai and Red Banana are very susceptible

Characterisation

These are isometric particles with a size of 20 mm diameter. The genome is single-stranded DNA. Each particle has six genomic segments. This virus is semipersistently transmitted by aphid *Pentalonia nigronervosa.*

Banana Bract Mosaic (BBMV)

Symptoms

Spindle shaped streaks can be seen on the pseudostem after removing the dead leaf sheaths and characteristic of bract mosaic on male bracts as well as on female bracts. Spindle shaped pinkish streaks can also be seen on underside of midrib, bootleaf, spathe and some times on peduncle. Varied symptoms expressions by different cultivars has been reported at NRCB. Mosaic symptoms, necrotic streak symptoms were also observed in cultivars of Pisang Awak group and Monthan group respectively. During July to February, the leaves show spindle shaped mosaic and on the corresponding backside of the leaf the thick spindle shaped waxy coating is conspicuous. Pseudostem necrosis, necrotic streaks on midrib and petiole are also observed on cv. Ney poovan. In Nendran, the leaves are oriented in such a way giving the appearance of "Travellers Palm" tree. Reduction in finger size has been noticed in all affected varieties. Mild mosaic symptoms are also observed on fingers and peduncle. Unusually long and too short peduncle have been noticed in cultivar Nendran. Extended female phase after a short male phase has been observed in some cultivars.

Susceptible cultivars: Ney Poovan, Nendran, Monthan, Poovan etc.

Characterisation

BBMV virus belongs to potyviridae group. Particles are flexuous rod shaped with

size of 750x12 nm. The genome is single stranded positive sense RNA. Most of the plant viruses belong to this group. This virus is non-persistently transmitted by the aphid, *Aphis gossipii and Rhopoloshipum maidis.* Almost all the poty viruses have been reported to be sap transmissible. But mechanical transmission has not yet been proved for BBMV. Typical inclusion bodies of pinwheel shape are found to be associated with the viral infection and this is a characteristic feature of all poty viruses.

Banana Streak (BSV)

Symptoms

It induces broken or continuous chlorotic streaks or spindle shaped patterns which are first chlorotic, then become necrotic, giving a black streak appearance. Necrosis also occurs on the midrib, petiole and leaf sheath especially in cv. Poovan. Some isolates induce cigar leaf and psuedostem necrosis, leading to plant death. Symptomless infection also occurs frequently which can be confirmed only by EM or sero-diagnosis tests. Cool months and other abiotic stresses are reported to induce prominent and severe symptoms. In India, abnormal bunch emergence and seediness have also been observed in cultivar Poovan. The most important characteristic of BSV is that, the symptoms are not uniformly distributed on the leaf. When BBMV and BSV both infect a Monthan variety, they induce necrotic streaks on fingers and cracking of fruits also observed. The susceptible varieties are Poovan, Robusta, Red banana, Grand Naine and Basarai.

Characterisation

This virus belongs to para retrovirus group, Badna (Baciliform DNA) virus. The size of particle is 130x30 nm. The genome is ds DNA with 4000 nucleotides. This virus integrate with the host genome. The citrus mealy bug, *Planococcus citri* found to transmit the virus in a semipersistant manner. Distribution of virus within the plant is not uniform.

Banana Mosaic/Infectious Chlorosis (CMV)

Symptoms

Mild or severe chlorosis, chlorotic streaking or flecking, mosaic symptoms on lamina, leaf distortions are the major symptoms. In India, especially in Maharashtra and Gujarat heart rot symptoms appear during winter seasons, Cigar leaf necrosis and pseudostem

rot are also the manifestation of CMV. Lockhart (1986) distinguished CMV infection from BSV, based on presence of midrib mosaic induced by the former. BSV induces severe chlorotic symptoms when the temperature is high, whereas CMV symptoms are suppressed at higher temperature. Using indicator host, dual infection of CMV and BSV can be distinguished.

Among all banana viruses, CMV is the only virus found to be transmitted mechanically to many indicator hosts. The indicator host produces conspicuous necrotic/chlorotic local lesions. This technique can be used for indexing the samples for CMV. *Chenopodium amaranticolor, C.quinoa, C.murale, C.album, Vigna unguiculata, Phaseolus vulgaris cv. prince* and *Nicotiana tabacum cv. Xanthi* are some of the indicator hosts producing necrotic local lesions. When a mixture of viruses are associated with banana CMV, they can be separated using this technique.

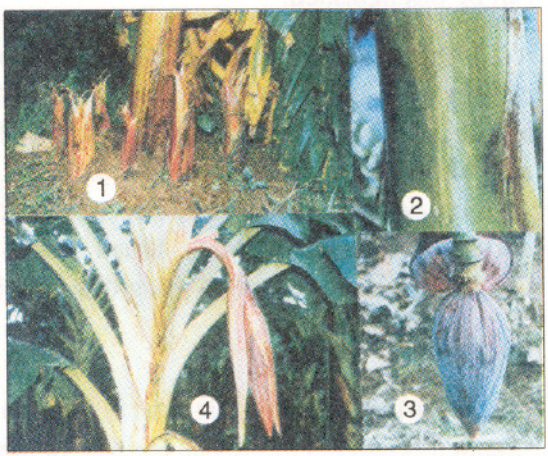
Characterisation

It is tripartite, isometric virus belongs to cucumovirus group. The genome is single stranded positive sense RNA. The size of the particle is 26 nm in diameter. The virus is transmitted by many aphids namely *Aphis gossypii, Rhopalosiphum maidis, R. prunifoliae* and *Myzus persicae* in a non-persistent manner.

VIRUS INDEXING

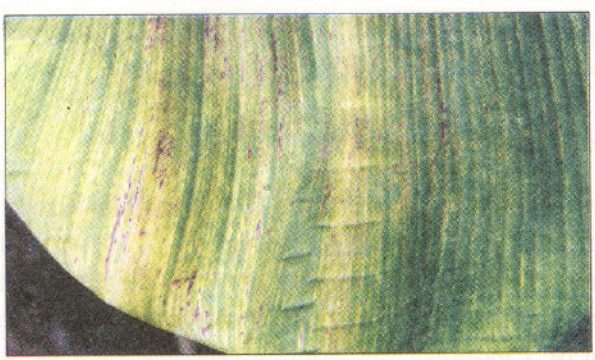
Effective techniques (Sero-diagnosis, Nucleic Acid probes, EM, Visual) have to be adopted to index the virus (minimum of 4 times). Virus indexing have to be carried out at various stages namely a) while selecting the mother plant, b) after complete plantlet development, c) during hardening and d) after hardening the plants should be kept in insect proof glass house for a period of 3 months. The indexing procedure has to be followed for all the banana viruses. If one of the progeny gives positive reaction for any of the viruses, the whole lot has to be discarded.

Banana Bunchy Top



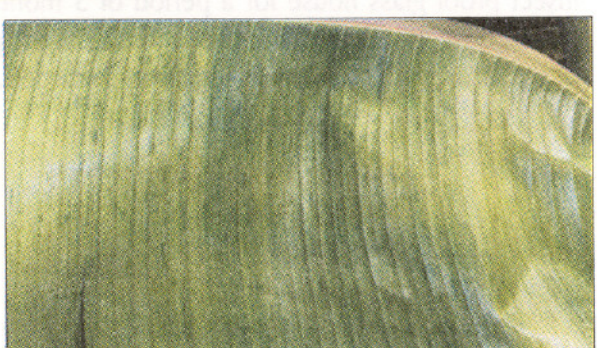
Bract Mosaic

1. Reddish streaks on suckers
2. Mosaic on pseudostem in Monthan
3. Mosaic on bracts
4. Traveller's palm appearance in Nendran



Banana Streak

Banana Mosaic (CMV)



While indexing it is important to select the type of plant parts to be indexed as the titre varies in different parts of the plants depending upon the type of virus. At National Research Centre on Banana, Trichy it was found that the BBMV concentration is much more in the bracts than leaf laminae and leaf sheath. During selection of mother plant itself, the male bracts can be observed for symptoms, as well as for the virus under EM, which would ensure freeness from virus. It is reported that BBMV was present in flower bracts which had no symptoms of the virus. Midrib of leaf should be used for indexing Banana bunchy top virus (BBTV). Hence even if the visual symptoms are absent, EM observation for flexuous rod particles would give confirm results. Some of the virus indexing techniques are discussed hereunder.

Indexing Techniques

Electron Microscopy

For the presence of the particles of virus, EM observations can be made. The size and shape of the particles can be observed under EM with leaf dip preparation. Except BBTV which occurs in very less concentration, BBMV (flexuous rod shaped), BSV (baciliform) and CMV (isometric tripartite) particles can easily be observed under EM. High concentration of flexous rod shaped, BBMV particles are observed from the infected bract and leaf sheath of cultivars Robusta and Nendran whereas a few, Baciliform, BSV particles are observed from infected Poovan samples with the use of ISEM technique. The mother plants should be checked for virus under EM.

Enzyme Linked Immunosorbent Assay (Elisa)

Among the serological diagnosis techniques, ELISA is the widely used technique to screen large number of samples against many viruses. This technique is simple, reliable, quick and consumes less time. In this method, the specific antibody produced against a particular virus is absorbed on to a solid polystyrene coat. The plant samples suspected to have virus is coated over it. If virus is present, it binds with the pre-coated antibodies. The enzyme linked conjugate having the specific antibody is poured in the wells, hence forming sandwich of two layers of the specific antibodies and a layer of the antigen, the virus in between. The enzyme, alkaline phosphatase/peroxidases, hydrolyse the substrate and forms a colour which can be read at 405 nm. The absorbance value is directly proportional to concentration of virus present in the sample.

membrane is floated/submerged in specific antiserum solution. Later the blotted membrane is submerged in the universal conjugate solution. Thereafter, the substrates, BCIP and NBT are added for final reaction which forms insoluble colour on hydrolysis. The density of the colour is directly proportional to virus titre. This method is most suitable for field level indexing, During survey the samples can be blotted and the blotted membranes can be sent to main diagnostic centres for rapid detection.

Western Blot

In this method, the coat protein of the virus is separated in SDS-polyacrylamide gel electrophoresis. Then the coat protein is electro-blotted on to Nitrocellulose membrane. The remaining procedure is followed as given in DIBA technique. This technique ensures that only coat protein reacts with antibodies. Any cross reaction with host proteins can easily be identified. The purity of antiserum can be confirmed in this technique.

Polymerase Chain Reaction(PCR) technique

When only a small quantity of virus occurs in host, it is very difficult to detect the virus. Hence using known short oligonucleotides of virus, the viral genomes present in host can be amplified exponentially (2^n) using this technique. After amplification using DNA or DNA probes, the amplified sequences can be detected. This technique is very useful for BBTV and BSV, because these two viruses are difficult to purify and also occurs in undetectable quantity in the host.

SHOOT TIP CULTURE

Shoot tip, which includes meristem with few attached leaf primordia is effectively used for *in-vitro* multiplication of banana. Stepwise procedure involved in *in-vitro* multiplication through meristem culture is discussed in detail.

Selection of mother plant

Careful selection of a mother plant is very important as hundreds of plants will be obtained from a single source plant. In general good mother plant should be true to type, with good vigour, growth rate and desirable fruit characters. In addition, sucker

should also be healthy with good appearance. Normally, three months old sword suckers is preferred, though peepers and buds are also used as explants.

In the field the sword suckers of 1.0m tall are identified and carefully extracted, without damaging the rhizome portion. Soil, roots, outer leaves are removed and trimmed to a size 7cm tall and 4cm wide. They are treated with 0.1% Mercuric chloride containing 0.1% Cetrimide and Streptocycline in a conical flask and brought to the laboratory.

Initiation/Culture establishment

In the lab, they are cut to a size of 2.5 cm x 2.5 cm x 2.5 cm and transferred into fresh solution of 0.1% cetrimide and streptocycline for 10 min. After this the trimmed suckers are treated with 0.1% mercuric chloride for 5 min. After removing from mercuric chloride solution, 3-4 washes are given with sterile distilled water to remove traces of mercury. They are taken out, drained off all the water before removing leaf sheaths. At this state care should be taken to limit the duration in mercuric chloride solution to only 5 min. All these operations in the lab are carried out under laminar air flow cabinet.

Size of the explant

It is very important to have an optimum propagation rate of healthy culture, for which size of explant plays a prime role. Though, very small explants is expected to produce disease free plants, mortality is very high. Use of bigger explant is also not favoured since it increases blackening and bacterial contamination. Expression of bacterial contamination may be immediate or during proliferation stage (latent contamination). Hence, most appropriate explant size is 1.5 cm^3. These explants show early signs of life and throw out more axillary buds. At times, when valuable explants especially those of germplasm are contaminated, decontamination can be under taken as they can tolerate the intensive decontamination treatment without much mortality.

The final explant contains 5-6 leaf sheaths with a growing meristem measuring 1.5 cm^3. In case of meristem tip culture, the explant consists of only the meristematic clone, excised carefully using a dissecting microscope. Meristem tip culture is used in decontamination of valuable accessions affected by viruses. Since the mortality of explants in meristem tip culture is high, shoot tip culture of indexed, virus free plants is resorted to

allow higher explant survival. It is placed in a semi solid medium, incubated at 26°C with an R.H. of 60-70% and 16 hr light cycle with fluorescent light of 1000-3000 lux. Proper lighting plays an important role as 1000 lux and below affects normal growth.

Culture Media

Optional growth under *in-vitro* conditions depends mainly on the nutrient medium, *In-vitro* maintained plants have to be provided with external source of food. Medium containing MS salt with additional levels of phosphate, organics and vitamins is generally used for banana. Details of the medium constituents is enclosed in Annexure.

Among plant growth regulators, cytokinin is very important in *in-vitro* multiplication of banana. Concentration of cytokinin determines the development pattern. 6-Benzyl Amino Purine (BAP) is widely used as it induces maximum shoot bud proliferation. Use of Auxin is not very essential, since rooting is not a problem in *in-vitro* banana culture. It can be seen that roots are produced even under normal BAP level after several subculture cycles. Auxin, when used, it should be used in small quantities. Ascorbic acid an antioxidant, at a concentration of 10mg/l is used in the medium to reduce blackening. Use of Myo-inositol at a rate of 100 mg/l helps in faster development and enlargement of buds.

After dissolving all the ingredients of the medium in distilled water, pH is adjusted to 5.8 with 1N NaOH. Agar at a rate of 7g/l is used to solidify the medium. Normally glass jars of 300ml capacity are used. Test tubes can also be used as they minimise contamination. But for mass multiplication jars are preferred owing to easy of handling, larger volume and easy cleaning. Medium is dispensed at 100 ml/bottle and sterilised by autoclaving at 121°C and a pressure of 1.05 kg/cm^2 for 20 min. Care should be taken to maintain proper pressure and time duration while autoclaving. Increase in duration or pressure eventually results sucrose charring, medium discolouration in addition to loss in activity of other essential ingredients.

After a week of initiation, explant turns green and swells up, showing signs of life. Leaf sheaths lift up gradually. By 3-4 weeks a single shoot may arise.

First subculture

First subculture is done after 4 weeks of inoculation. By this time, explants enlarge to about double the original size. The blackened surface is scrapped off, and corm base is reduced to 0.5cm thickness. Reducing the thickness of basal corm tissue decreases blackening, eventually enhances the chances of proliferation of non-meristamatic tissue outgrowing the meristamatic tissue. Any growing apical shoots, is cut and the growing tip damaged by giving two longitudinal cuts perpendicular to each other. Here care is taken to keep the base intact, so that the explant remains as a single unit. Cutting is done to would the shoot apex which contains the growing apical meristem. This activates the axillary buds by suppression of apical domination. Later the explant is transferred into fresh medium the again incubated for about 4 weeks.

Second subculture

By the end of first subculture, shoots may be seen with 1-3 side buds. The blackened surface is scrapped off, and vertical cuts given in SC-1 are extended till the corm base, so that four quardrants are obtained. Some times, one or more of these quadrants turn black and showing no signs of axillary buds. Quadrants with axillary bud(s) showing activity are isolated. and by the end of second subculture cycle, the buds enlarge to form clumps. In the subsequent subcultures, these buds are sub divided, so that more number of buds are produced. This subculture cycle is repeated at 4 weeks interval to increase the proliferation rate.

Rate of multiplication

Multiplication rate mainly depends on the genomic group to which the explant belongs and the concentrations of growth regulators. Normally, initiation medium contains high BAP levels upto 6 mg/l. In the subsequent subcultures, it is reduced to 3 mg/l. The number of cycles of subculture depends on the genomic group.

Diploids with AA genomic group like cultivar Rose, Pisang Lilin multiply profusely but put forth weaker plantlets. Triploids with AAA genomic group under *in-vitro* conditions, can tolerate high level of BAP and induce large number of shoots. Corm formulation is not observed in this group. In acuminata-balbisiana hybrids like in Rasthali, Pachanadan and Poovan, clumps are formed from 2nd subculture onwards and these clumps increase in number and size with the advance of number of subcultures. With reduced BAP levels, they revert to shoot formation very quickly. In Rasthali, good proliferation in large number of clumps containing minute buds, posting problems in regeneration. Hence use of a medium levels of BAP is recommended. In groups with more of *Balbisiana* genome (ABB) like in Karpuravalli, Saba, Bluggoe, bud clumps are formed from 2nd subculture onwards and the size increases with the subculture. Big corms are obtained by 5th or 6th subculture under normal BAP levels. To avoid this, BAP level should be reduced to normal after initiation and reduced to 1/10th after 5-6 subcultures and is continued for 2-3 cycles for shoot development.

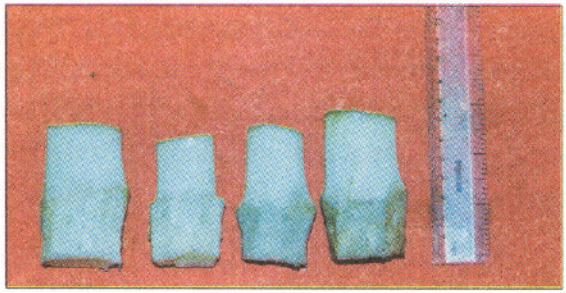
Blackening of the explant and the media is seen during culture initiation and subsequent two subcultures. Blackening is caused by oxidation of phenolic compounds in wounded tissue and is more in cultivars with 'B' genome. Nendran shows comparatively more blackening. To overcome this following precautions have to be taken.

* Adding ascorbic acid to the medium as an antioxidant
* Maintaining very little corm tissue of the explant
* Frequent transfer of explants to fresh medium after every 3-4 weeks
* Adding activated charcoal to the medium

Decontamination

Contaminated cultures are though best discarded, decontamination is resorted, if the material is a valuable germplasm. For bacterial decontamination, antibiotics are used in the medium, too high a concentration of antibiotic in the medium causes growth aberrations. While too low a concentration of antibiotic in the medium proves ineffective in decontaminating bacteria. An optimum concentration of 0.1% of streptocycline is

Suckers for extraction of shoot tip



Trimmed suckers ready for shifting to lab from field

Ready for 3rd subculture

Well rooted plants in rooting media ready for *ex-vitro* hardening

found to be effective in decontaminating a wide bacterial spectrum. 1 ml of 0.1% sterile solution is streptocycline is added to an autoclaved medium bottle just before solidification, when the temperature is around 30-40°C. This is done under aseptic conditions. Contaminated cultures are transferred into the antibiotic medium and the subculture cycle repeated till the plants are shifted out in *in-vitro* conditions.

Regeneration

After 5-6 subculture cycles, proliferating cultures are shifted to a medium with 1/10th in original BAP concentration. This induces shoot developments. Two cycles of subculture in low BAP medium are required for regenerating plantlets. Once the shoots are produced, they are transferred to rooting medium containing 1/2 MS+1mg/l of IBA+0.25g of activated charcoal. Roots start appearing in about a weeks time. By one month, rooted shoots of 6-10cm tall with well ramified roots are obtained and are ready for shifting for hardening.

PROBLEMS ENCOUNTERED DURING *IN- VITRO* MULTIPLICATION

There are many practical problems encountered during *in-vitro* multiplications as the plant is forced to grow in artifical conditions out of its natural habitat. They may be classified as,

Insoluble precipitates

The main cause for precipitation of medium is due to the complexes formed between calcium, magnesium and phosphate. To prevent this, a sequence is maintained while adding chemicals like nitrogen compounds followed by magnesium compound, calcium compound and finally phosphate compound. Of all these, calcium compound is dissolved separately and then added rather than as a salt.

Blackening

Blackening is mainly due to oxidation of phenolic compounds exudated from the cut surfaces. Explants and media generally blacken during culture initiation and subculturing. But this varies with variety, Pisang Awak (ABB) and plantains (AAB) respond very strongly by turning black. Many valuable germplasms are lost due to the blackening. To avoid this problem, the following precautions are required to be taken.

* Explant be dipped in antioxidants like ascorbic acid, citric acid or cysteine, prior to inoculation.
* Addition of anti-oxidants like ascorbic acid or activated charcoal (25 mg/ l) into the culture medium.
* Increasing the frequency of sub-culturing to weekly intervals. After frequent subculturing, frequency of subculturing may be decreased from 1 week to 3-4 weeks.

Sometme increased cytokinin concentration of facilitate proliferation also cause tip blackening. Before tip completely blackened, subculturing is carried out with cutting off of the blackening tip.

Contamination

Contamination is not only a problem in cultures but also in stock solutions like vitamins and growth regulators. Contamination can occur at any point of the whole sequence of operation. It may occur by spore transfer or by contact with incompletely sterilized media, equipments, solutions, and surfaces. Contamination sources are mainly bacteria, yeast or fungi. Indentification keys are as follows.

* Bacterial contaminations are recognised by dull shiny spreading growth on media and turbidity in liquid media and at times emitting off odour.
* Fungus is identified by their cottony clumps on a solid media and like balls in liquid media.
* Moulds contamination is often mistaken for fungus contamination, it bears gray-green fruiting bodies after few days of appearance.
* Yeast grown often appears as a heavy 'milky' turbidity in liquid medium and has distinctive off odour.

To avoid microbial contamination, focus should be to keep the incidence at absolute minimum of contamination which appears in a series of tubes inoculated on the same way, mainly owed to improper sterilization. Thus care should be taken for regular autoclaving of dissecting and inoculating instruments. Contaminated container should be autoclaved before opening it for cleaning. But incase of valuable material contaminated by bacteria may be rescued by transferring it to a medium containing antibiotics. Prolonged use of antibiotics may lead to sudden inherent changes in plant structure hence it is used where alternative means of eliminating contamination have failed.

Somaclonal variation

Variation among plants generated from cultured cells or tissues is defined as somaclonal variations which are heritable in nature. They occur at all levels of *in-vitro* culture viz. callus, suspensions, adventitious shoots, somatic embryos and also in regenerated plants. Due to somaclonal variations, genetic integrity of the material is lost with a change in both qualitative and quantitative characters at morphological, cytological, biochemical and molecular levels. There is a great variation with respect to varietal susceptibility to somaclonal variants.

Reported phenotypical somaclonal variations among banana and plantain cultivars observed in shoot tip culture are listed below.

Plant stature

a. Dwarfism
b. Thin and sickly looking tall plants

Pseudostem

a. Abnormal pseudostem pigmentation, with and without blotching

Leaves

a. Variation in leaves
b. Twisted and crinkly leaves
c. Narrow and drooping leaves
d. Leaves with unusually wavy margins

Inflorescence

a. Abnormal bunch orientation unlike the parent plant
b. Small, narrow elongated male bud or bloated male bud
c. Absence of male bud and its reversions.

Bunch

a. Small bunch with short fingers
b. Variation in hand and finger orientation on the bunch
c. Persistency or deciduous nature of floral bracts
d. Hairyness on the bunch peduncle and fruit

e. Twisted fingers
f. Warty fruits with ugly eruptions.

Acuminata triploids, especially Cavendish sub group succumbs to this variation quite often leading to dwarf types. In some cases plantains revert often from 'male budded sport' to 'budless sport'.

Better method to minimise somaclonal variation is periodical inspection and roguing. This includes even while subculturing the plantlets. In nursery stage also utmost care is required to be taken to eliminate suspected variant. At the same time, many important clones of commerce have arisen as spontaneous mutants. For example, Highgate is a dwarf mutant of Gross Michel (Simmonds, 1966) and Gandevi selection, a tall mutant of Dwarf Cavendish.

HARDENING TECHNIQUES

Tissue culture plants are produced in a closed, sterile environment and grown in a nutrient rich artificial medium under controlled conditions. At this stage, plants are made to shift from largely heterotropic to fully autotropic nutrition with an ability to bear abiotic stresses like low humidity, high light intensity, water stress etc. 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.

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 reduce survival rate. Temperatures below 10°C and above 40°C should be avoided. The package should be protected from direct sunlight and be kept in an upright position. If the transit time exceeds 48 hrs, it is desirable to occasionally expose the flasks to light, if possible.

Tissue cultured plantlets should 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°C - 35°C is adequate. Tissue cultured plantlets which are ready for transfer to polybag, can usually be kep in the net pot for another 2-3 weeks. Microbial contaminations should be monitored upto 2 weeks and infected plantlets should be destroyed. For best results plants should be measuring 7-10 cm. tall with strong root system. Most of the commercial tissue culture laboratories supply primary hardened plantlets in net pots which are cheaper.

Primary Hardening

In-vitro grown plants are heterotropic, since all the requirements for its multiplication and growth are provided externally and artifically. It is necessary for plants to get acclimatized to *ex-vitro* conditions to become autotrophic. Therefore, plants will be gradually exposed to conditions of decreasing humidity and increasing light intensity. *In-vitro* grown plants are delicate, have less developed cuticle, poor stomatal activity, limited mesophyll development and with plenty of intracellular cavities. Hence they have to undergo anatomical and physiological changes for their establishment in green house conditions, which is done gradually. From the culture room, culture bottles will be shifted outside and kept under shade for 4-5 days. This decreases the relative humidity and promotes better cuticular wax formation under less cuticular transpiration. One day before of planting, water (100ml) is poured into the culture bottle and kept under shade. This will make the plants hydrated to sustain the loss of water to some extent, after planting. On the day of planting, plants are removed from the container, washed gently to remove the traces of agar sticking to the roots. If roots are long, they are trimmed to about 6-7 cm length, dimmed in 0.1% Bavistin solution and planted in plastic trays containing mixture of cocopeat and soilrite in equal proportion. *In-vitro* grown plants are completely sterile. Hence, treating with fungicide is necessary before planting. In places of occurrence of *Erwinia,* it is adviced to treat the potting mixture with 0.1% Emisan, before filling into the microtrays. Watering is done immediately after planting till the saturation point. Bigger leaves are trimmed and trays are covered with low height polythene tent so as to maintain required high humidity and low light intensity. Occassional water spraying is done only to the leaves. The plants are kept in this condition for 4-5 days. During this period polythene tent is kept open for few hours in a day and gradually this duration is increased. By about 15 days plantlets will be ready for shifting to secondary hardening.

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 1 2:1, 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 helps to prevent the incidence of bacterial wilt. Addition of neem oil cake at the rate of 50g per kg of potting mixture helps reducing nematode incidence from net pot to poly bags during hardening.

Bag filling

The potting mixture should be filled to the brim in black polythylene bags (1 50 gauge) of size 1 5cm diameter and 30cm height. If bag size is reduced, the time taken for secondary hardening would be more than 45 days.

Plantlet transplantation

Plantlet transplantation into 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. The bags should be watered a day prior to transplanting. A small planting hole of about 5 cm deep can be made. Plant the plantlets into the hole in upright position and cover with potting mixture and press gently around the plantlet. Irrigate the transplanted plants on the same day. The next day the bag is drenched with 1 00ml of Emisan-6 at 0.1% to guard against fungal and bacterial contamination. Also 3 grams of carbofuran 3G is added to prevent nematode infestation.

Watering 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 the water balance until several days after transplanting. Use of 50% shade net and regular misting of water can increase necessary environment for growth

and hardening. Ideally the relative humidity should remain above 90% for atleast a week. When plants have developed several leaves, they are irrigated twice every morning and evening.

Establishment in nursery

The polythene bags with plantlets are best arranged in double rows which saves space and still allows for maintained in proper leaf development on either side of the open secondary hardening stage alleys between rows. Watering should preferably be daily and a must during the first two to three weeks. Use of low head sprinkle 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 50 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. 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 a range from 15-25^0 C, with an optimum of 23-25^{0}C should be maintained.

Manuring

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

Individual plants in net-pots

Tissue culture plant ready for shifting

Transplanting in polybag

Secondary hardening in net house; plants kept in double rows

A high standard of hygiene is essential to minimize the risk of damage by pests and diseases. The following pest 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, remaining 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 become black. Cross section of corm will exhibit yellow brown soft rot extending upto the growing point. Occasionally the bacterium may attack the neck region and the leaf symptoms will appear from the peripheral leaves towards the centre.

To control the malady, the soil in the bage 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 emisan 6. Spraying streptocyclin sulphate to 3 weeks old seedling 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 oxy chloride or 0.1% dodine 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 plants are more susceptible to nematodes like root knot nematode, lesion nematode, burrowing nematode, spiral nematode, as the roots are very tender. It is very essential to take up nematicidal application from the beginning. Three gram of carbofuran 3G is applied at planting in the bag at 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 mycorrhize (VAM) per plant would be beneficial for better root development, besides reducing the nematode population.

Erwinia soft rot
in secondary hardening

Bacterial rotting
of *in-vitro* plants

Infestation of rootknot
nematode

FIELD PLANTING AND INITIAL MANAGEMENT

Plants should be kept in the nursery 40-60 days until they are 20-30 cm tall with 3-5 broad leaves. Weaker plant should be separated and be 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 by removing the nursery bags. Such wider spacing will prevent the plants from growing too all 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 bag level 1-2 months before planting. Field planting should be done earlier 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 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 be removal of the polythene bag (with out its bottom) by 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 practice in the area. The first new leaves should be formed within 2-4 weeks planting in the field. Plants may be kept watered in the field soon after planting as young micropagated plants cannot withstand dry weather and heat. While planting in the field, 10g of carbofuran 3 G is applied per plant. Within 3 days of planting, drench the soil around the plants with 500 ml of 0.5 per cent emisan 6 to guard the plants against bacterial soft rot to which the tissue cultured plantlets are highly prone.

It is necessary to be assured that the tissue cultured plants are 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.

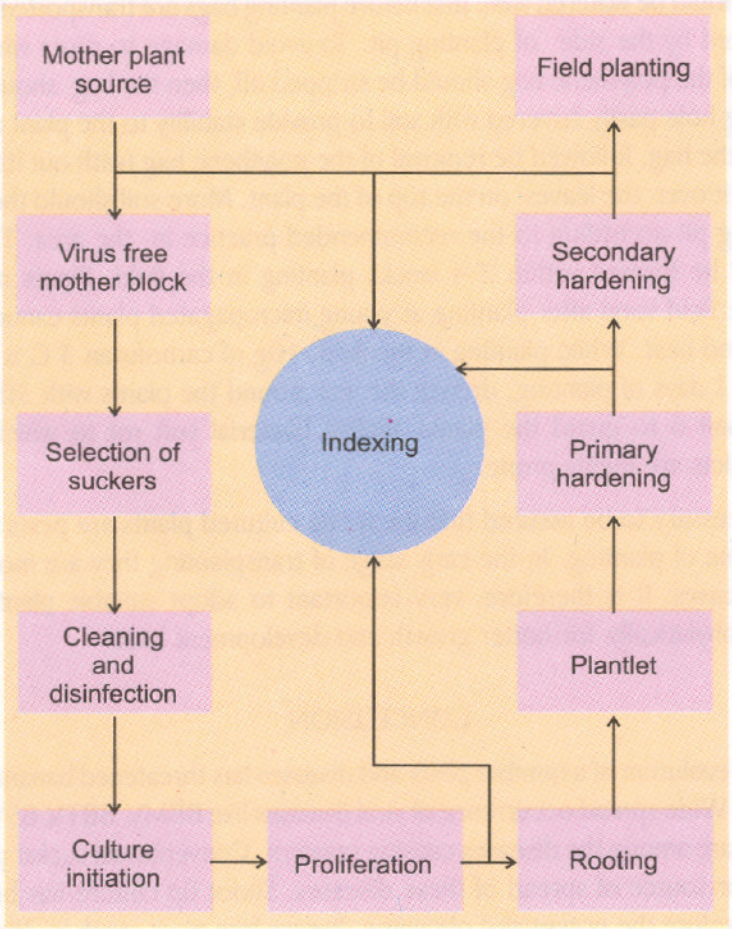
CONCLUSION

The co-evolution of a number pests and diseases has threatened banana cultivation at global level. Wide spread occurrence of viral diseases like BBMV, BBTV, BSV, Infectious Chlorosis etc. are among the diseases causing concern. Conventional sucker propagation has been major source of spread of these diseases. Shoot tip culture has been a novel approach to explore the potentialof obtaining disease free plant stock by eliminating in-

built viruses. But the failure of shoot tip culture in completely eliminating virus has highlighted the necessity of 'Virus Indexing' at every step to maintain the clonal health at the user level. The steps given in schematic diagram ensure the production of virus free healthy plants through shoot tip culture. Careful selection of mother plants and continuous use of disease free planting materials will greatly help in increasing the production and productivity apart from rehabilitating the infected area. But this becomes reality with true commitment from the commercial entrepreneurs to distribute the farmers only the disease free and authentic planting material after indexing. With this, the target of achieving 25 million tonnes by 2020 AD may be achieved well in advance.

———— × ————

Schematic Diagram of *in vitro* multiplication of virus free Banana



USEFUL REFERENCES

Cronauer, S.S. and A.D. Krikorian.1984. Rapid multiplication of bananas and plantains by *in-vitro* shoot tip culture. *HortScience,* 19:234-235.

Diekmann, M and C.A.J. Putter (Eds), 1996. *Musa Spp. FAO/IPGRI Technical Guidelines for the safe movement of Germplasm.* No:15. pp 23.

Hwang, S.C., Chen C.L., Lin J.C. and Lin H.L. 1984. Cultivation of banana using plantlets from meristem culture. *HortScience,* 19:231-233.

INIBAP, 1993. Biotechnology Application for Banana and Plantain Improvement. Proceedings of a Workshop held in San Jose, Costa Rica, in Jan., 1992. pp. 251.

Israeli, Y., Lahav, E. and O. Reuveni. 1995. *In vitro* culture of banana. In: Banana and Plantain by Gowen, S.R. (ed.) *Chapman & Hall.* pp 1247-78.

Jarret, R.L. 1986. *In vitro* propagation and genetic conservation of banana and plantains. In: *IMPGR Advisory Committee on In vitro Storage. Report of the Third meeting (Appendix). IBPGR, Rome,* Italy.

Krikorian, A.D. and S.S. Cronauer. 1984. Aseptic culture techniques for banana and plantain improvement. *Econ. Bot.,* 38:322-331.

Simmonds, N.W. 1962. The Evolution of edible Bananas. *Longman. London, pp.* 170.

Singh, H.P. and S. Uma. 1996. Banana Cultivation in India. Directorate of Extension. Min. of Agric., New Delhi. pp. 102.

Vuylsteke, D.R. 1989. Shoot tip culture for the propagation, conservation and exchange of Musa germplasm. *IBPGR, Rome.* pp 56.

Wu, R.Y. and H.J.Su, 1990. Purification and Characterization of Banana bunchy top virus. *J. Phytopathology.* 128: 153-160.

ANNEXURE

Medium containing Murashige and Skoog (MS) mineral salt mixture with organics, vitamins and hormons is well suited for banana micropropagation. The detailed procedure of preparing MS medium is given, which is used for successful multiplication of banana.

Medium composition for *in-vitro* multiplication of banana (in mg/lit)

NH_4NO_3	1650	Glycine	2.0
KNO_3	1900	Thiamine HCl	0.1
$CaCl_2.2H_2O$	440	Nicotinic Acid	0.5
$MgSO_4\ 2H_2O$	370	Pyridoxine Hcl	0.5
$K\ H_2PO_4$	400	$FeSO_4.7H_2O$	27.8
H_3BO_3	6.2	Na_2 EDTA. $2H_2O$	37.22
$MnSo_4$	16.9	Ascorbic Acid	10
$ZnSO_4.2H_2O$	8.6	BAP	3
KI	0.83	Meso Inositol	100
Na 2MoO4. $2H_2O$	0.24	Sucrose	30g
$CaCl_2.6H_2O$	0.24	Agar	7g
$CuSO_4\ 5\ H_2O$	0.025		

Stock Preparation

The above composition represents the quantity of the constituents in working solution. In practice, it is easier to prepare stocks and store them in a refrigerator, for aliquot and use as required.

Macro and Micro Nutrients

Stock A

1.	Ammonium Nitrate	33g
2.	Potassium Nitrate	38g
3.	Magnesium sulphate	7.4g
4.	Potassium Dihydrogen Phosphate	8.0g
5.	Boric Acid	0.124g
6.	Manganese sulphate	0.338 g
7.	Zinc sulphate	0.172g
8.	Calcium chloride	8.8g

Dissolve all the nutrients from 1 to 7 in about 500 ml of double distilled water, one after another. Care should be taken to dissolve one nutrient completely before adding the next one. Dissolve calcium chloride separately in about 200 ml of double distilled water as it precipitates with other salts. Mix both the solution throughly and make up the volume to 1000 ml and store under refrigerated condition.

Stock B

Potassium Iodide - Dissolve 1.66g in 100 ml distilled water and store in fridge.

Stock C

Prepare 0.5% solution of Sodium Molybdate in double distilled water and store in refridgerator.

Stock D

Prepare 0.5% Cobaltous Choloride solution using double distilled water.

Stock E

Make 0.5% Copper sulphate solution using double distilled water.

Stock F

Glycine	50 mg
Thiamine hydrochloride	2.5 mg
Nicotinic acid	12.5 mg
Pyridoxine Hydrochloride	12.5 mg

Dissolve all the above mentioned nutrients in about 100 ml of double distilled water, make up the volume to 250 ml and store in fridgerator.

Stock G

(a) 1.39 g Ferrous sulphate in about 200 ml of hot double distilled water

(b) Dissolve 1.8625 g of disodium EDTA in about 200 ml of double distilled water

Mix (a) and (b) make up the volume to 500 ml, store in amber coloured bottles under refrigerated condition.

Stock I (Growth regulators)

Dissolve 50mg of BAP in 3-5 ml in NaOH. Take care to dissolve it thoroughly. Add double distilled water and make up the volume to 100 ml. Store under refrigerated condition.

To prepare 1 litre of multiplication medium , add the the following volumes of respective stocks to about 800ml of double distilled water in an erlonmeyer flask.

Stock A	:	50 ml	Stock B	:	1 ml
Stock C	:	0.96 ml	Stock D	:	0.1 ml
Stock E	:	0.1 ml	Stock F	:	10 ml
Stock G	:	10 ml	Stock H	:	2 ml
Stock I	:	6 ml			

Add 100 mg of Myo-inositol and 30 g of Sucrose. Dissolve it thoroughly. Adjust the pH to 5.8 by using IN NaOH. Make up the volume to 1000 ml. Add 7g agar and boil till it clarifies, dispense 50ml into each glass jar, screw the cap tight and autoclave at 121°C (15 psi) for 15 min.