



Banana Tissue Culture in India

A Success Story





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Foreword

Banana and plantains are the second largest food-fruit crops of the world and produced in the tropical and subtropical regions of mostly the developing countries of Asia Pacific. It has been observed that in recent times horticultural crops have taken over food grain production in India and this has been possible because of focused effort on increased production of fruits and vegetable crops through various government policies, on availability of quality seed/planting material, incentives in form of subsidy on production of horticultural crops and better agronomic practices. Among fruits, banana is the most consumed and is highly nutritious fruit crop with high dietary fibres, vitamins and minerals. Its cultivation in India has been transformed because of introduction of tissue cultured quality planting material of Cavendish banana variety Grand Naine or G9.

The cultivation of tissue cultured banana plants has been increasing and providing higher income to farmers and it has led to banana revolution in India. A large part of credit goes to Department of Biotechnology (DBT) which has taken much needed initiatives for quality control in production of tissue cultured plants by commercial units in India. Heartening to note that DBT has established National Certification System of Tissue Cultured Raised Plants for this purpose.

APAARI in 2011 has brought out status papers on *Micropropagation for Production of Quality Banana Planting Material in Asia-Pacific*. As banana fruit production using tissue cultured plantlets has been increasing in the region, a further need was felt to compile all the information available on different aspects of banana cultivation including tissue cultured banana in India and publish as **Success Story on Banana Tissue Culture in India**. It is expected that the publication will serve as a case study for most of the banana growing countries of the region and may foster collaborations among the relevant partners in research and development apart from serving as a reference for policy makers.

I must compliment the authors Drs VK Baranwal, Reetika Kapoor and Shiv Kant Shukla for making a meticulous effort to put together information on different aspects of banana cultivation, production and certification of tissue cultured banana and success stories of farmers who have adapted cultivation of tissue cultured banana. I also thank Dr Rishi Tyagi for conceptualising and ensuring this important publication.



Ravi Khetarpal
Executive Secretary, APAARI

Preface

Banana belong to family *Musaceae*, is among the world's most popular fruit and a staple food crop for millions of people. Banana is native to Southeast Asia and is widely grown in the tropical, subtropical and coastal regions of India. Bananas are the most widely consumed fruits, being economical and also associated with a large variety of health benefits. It is a nutritional powerhouse, packed with essential vitamins and minerals required for the proper functioning of body. The total annual world production of banana is estimated at 113.91 million tonnes. India leads the world in banana production with an annual output of about 30.47 million tonnes, contributing about 26.7% in world pool of banana production (FAOSTAT, 2017).

In India, banana occupies 13% area among the total area under fruit crop (NHB, 2017). It is a vegetatively propagated crop, also being cultivated through tissue culture. Farmers predominantly use suckers as the planting material. However, some of the major limitations with conventional sucker planting technique are the presence of disseminating pests and diseases, high cost of suckers, low multiplication rates, variation in age and size of crops and unavailability of uniform suckers at the time of planting. Banana cultivation by suckers from a diseased mother plant is the main cause for spread of viral diseases in banana. Viruses are among the main constraints that severely affect the banana yields. In the last few years, advances in tissue culture production have made a great impact on the cultivation of banana crop. It is a superior technique over traditional method of sucker-propagated banana production in terms of optimal yield, uniformity, disease free planting material and true to type plants. Tissue culture (TC) banana cultivation gives more freedom as TC banana plantlets can be planted any time of the year except in extreme cold weather conditions. In India, a programme on certification of TC plants was conceived and initiated by Professor Anupam Varma of Indian Agricultural Research Institute (IARI), New-Delhi and Dr Renu Swarup of the Department of Biotechnology (DBT), Government of India. A National Certification System for Tissue Culture Raised Plants (NCS-TCP) was established by DBT in 2006 to promote the growth of TC industry in India. DBT was authorized as the Certification Agency for the purpose of certification of the tissue culture-raised plants for freedom from viruses up to laboratory level and regulating its genetic fidelity. Banana crop is infected by four important viruses *viz.*, banana bunchy top virus, banana streak Mysore virus, cucumber mosaic virus and banana bract mosaic virus and TC does not always ensure the elimination of viruses. Virus indexing of the mother culture and the subsequent batch of TC plants is, thus,

of utmost importance. Currently, under NCS-TCP, four accredited test laboratories (ATLs) and two Referral Centres (RCs), one designated each for virus indexing and genetic fidelity testing have been recognized to cater to the demand of testing and certification of plants produced by recognized Tissue Culture Production Facilities (TCPFs). Management Cell has also been established at Biotech Consortium India Limited, New-Delhi, India (BCIL) for managing and coordinating various activities of NCS-TCP. The main objective of NCS-TCP is to ensure the production and distribution of disease-free and genetically uniform plants to the farmers, thus, increasing the agricultural productivity. Under NCS-TCP, 28.607 million (TC) banana plants produced by the Indian tissue culture companies have been certified in 2016-17. Though, the initial cost of TC plants is much higher than the conventional suckers, farmers in India are adopting tissue culture banana (TCB) owing to the advantages in terms of better quality, uniformity, freedom from diseases, high yield and good market price. This has led to substantial increase in income of farmers who have adapted cultivation of TC banana. This compilation has made an attempt to put together different aspects of banana cultivation, production and certification of tissue cultured banana and success stories of farmers who have adapted cultivation of tissue cultured banana.

Authors

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We also appreciate the contribution of Mr Nishant Srivastava, Senior Research Fellow under Referral Centre for Virus Indexing, NCS-TCP for providing suitable photographs and figures related to this document.

Finally, we would like to thank all those who are directly and indirectly involved in the tissue culture of banana in India and without whom this success story would not have been possible.

Acronyms and Abbreviations

ABSP	Agricultural Biotechnology Support Project
APAARI	Asia-Pacific Association of Agricultural Research Institutions
APCoAB	Asia-Pacific Consortium on Agriculture Biotechnology and Bioresources
APEDA	Agricultural and Processed Food Products Export Development Authority
ATL	Accredited Test Laboratory
BBrMV	Banana Bract Mosaic Virus
BBTV	Banana Bunchy Top Virus
BCIL	Biotech Consortium India Limited
BSGFV	Banana Streak Goldfinger Virus
BSIMV	Banana Streak Imove Virus
BSMYV	Banana Streak Mysore Virus
BSOLV	Banana Streak Obinol'ewai Virus
BSVNV	Banana Streak Vietnam Virus
CMV	Cucumber Mosaic Virus
CPRI	Central Potato Research Institute
CQ-TCP	Certificate of Quality for the Tissue Culture Plant
DAS-ELISA	Double Antibody Sandwich Enzyme Linked Immunosorbent Assay
DBT	Department of Biotechnology
ELISA	Enzyme Linked Immunosorbent Assay
FAO	Food and Agriculture Organization of the United Nations
FAOSTAT	FAO Statistical Databases
FYM	Farm Yard Manure
G9	Grand Naine
ha	Hectare
IARI	Indian Agricultural Research Institute
IC-PCR	Immuocapture Polymerase Chain Reaction
IISR	Indian Institute of Sugarcane Research
kcal	Kilocalories
MOP	Muriate of potash
MT	Metric Tons

N, P and K	Nitrogen, Phosphate and Potash
NC	Negative Control
NCS-TCP	National Certification System for Tissue Culture Raised Plants
NHB	National Horticulture Board
NIPB	National Institute on Plant Biotechnology
NRCB	National Research Centre for Banana
PC	Positive Control
PCR	Polymerase Chain Reaction
RC	Referral Centre
RCA	Rolling Circle Amplification
RPA	Recombinase Polymerase Amplification
RT-PCR	Reverse Transcription Polymerase Chain Reaction
RT-RPA	Reverse Transcription Recombinase Polymerase Amplification Assay
SPB	Sucker propagated banana
TC	Tissue Culture
TCB	Tissue Culture Banana
TCCA	Tissue Culture Certification Agency
TCPFs	Tissue Culture Production Facilities
UAS	University of Agricultural Sciences
USDA	United States Department of Agriculture
VSI	Vasantdada Sugar Institute

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Executive Summary

Banana is a highly nutritious and important fruit crop produced by the tropical and subtropical countries of the world. India ranks first in the area and production of banana in the world. Traditional methods of cultivation involve the propagation of banana by vegetative mode through suckers. Production through suckers is limited by low multiplication rates, non-uniform growth, low productivity and the spread of various banana pests and diseases. The use of commercial tissue culture (TC) technology for mass production of banana varieties can address these problems to a great extent. In India, banana cultivation is gaining importance because of the planting of TC plantlets, which commenced during the mid 90's. In 2006, the Department of Biotechnology (DBT), Government of India, established the National Certification System for Tissue Culture Raised Plants (NCS-TCP) for the production and distribution of certified disease-free and genetically uniform tissue culture plants to the farmers. The Certification system is the first of its kind in the world that will significantly contribute to the increased agricultural productivity. At present, 82 commercial Tissue Culture Production Facilities (TCPFs) have been recognized under the NCS-TCP for this purpose.

This document on the 'Success story of Banana Tissue Culture in India' provides detailed information on banana, its different varieties grown across India, cultivation practices, various constraints faced in the conventional propagation of banana and how these can be overcome by the use of banana TC plants. Of the various varieties of banana grown in India, Grand Naine (G9) is the most preferred and commonly cultivated variety using tissue culture. Nearly, 5 million TC plantlets of G9 are planted in about 17% of total cultivated banana area in India every year. The journey of certification of banana TC plants in India has been discussed. Production of TC plants *viz.*, mother plant selection, initiation, multiplication and hardening are briefly described. Protocols used for virus indexing and genetic fidelity testing for successful implementation of certification system has been provided in detail. The molecular and serological based methods standardized under NCS-TCP for virus indexing of the four major viruses infecting banana have been described. Serological tests include ELISA based testing while molecular based tests include Polymerase Chain Reaction (PCR) and reverse transcription PCR (RT-PCR). Further attempts have been made to develop rapid and simplified virus indexing protocols useful for resource constrained laboratories. The tests for genetic fidelity and virus indexing are being currently performed at four accredited test laboratories (ATLs)

and two Referral Centres (RCs), recognized under NCS-TCP. Approximately, 28 million TC banana plants produced by the Indian tissue culture companies have been certified in 2016-17. The various challenges encountered during certification procedure of banana TC plants have been discussed with reference to emergence of new diseases, particularly the occurrence of Fusarium wilt caused by the soil-borne fungus *Fusarium oxysporum* in G9 cultivar, the production and distribution of plantlets, technology transfer for TC production and adoption of the technology by the farmers.

The 'banana tissue culture propagation technology' has become a popular technology for supply of disease-free quality planting material and has great scope for wide adoption by farmers. The TC technology has been profitable to the farmers and hence more farmers are opting for cultivation using TC banana plants. Success stories on cultivation of banana TC have shown that TC banana cultivation has increased the income of farmers many folds. In view of uniform and good quality of banana there is huge potential of banana export which is being currently dominated by Maharashtra state. A flow chart of export process shows that it requires harvesting of uniform banana, proper packaging, storage, transport and delivery to destination country and the same need to be adopted in many states on a larger scale. The export and economics of banana TC plants are indicative of occurrence of silent banana revolution in India.

1 Introduction

1.1. Banana

Banana is the oldest and commonest fruit known to the mankind. It belongs to genus *Musa* (family *Musaceae*), which is cultivated primarily for food and secondarily for the production of fibre used in the textile industry. Some of the varieties are also cultivated for ornamental purposes. In fact, every part of banana plant is used in some or the other form by human beings. Banana originated in Southeast Asia, in the jungles of Malaysia, Indonesia or the Philippines where many varieties of wild bananas still grow today. Africans are credited to have given the present name, since the word banana would be derived from the Arabic language for 'finger'. The Africans started its trading internationally by the end of the fourteenth century (<http://www.bananalink.org.uk/all-about-bananas>). Banana is grown in the tropics and it is consumed in most part of the world. Depending on their genomic constitution, the scientific names of bananas are *Musa acuminata*, *Musa balbisiana* or hybrids of *Musa acuminata* × *balbisiana*.

Bananas are vigorously growing monocotyledonous herbaceous plants. The banana plant is a gigantic herb that springs from underground stem rhizome and attain up to 15 meters of height. The cultivars vary greatly in plant and fruit size, plant morphology, fruit quality and disease and insect resistance. The trunk is composed of the basal portions of leaf sheaths and is crowned with a rosette of 10 to 20 oblong to elliptic leaves that sometimes attain a length of 3.0-3.5 metres and a breadth of 65 cm. A large flower spike, carrying numerous yellowish flowers protected by large purple-red bracts, emerges at the top of the false trunk and bends downward to become bunches of 50 to 150 individual fruits, or fingers. The individual fruits, or bananas, are grouped in clusters, or hands of 10 to 20 in number. After a plant has fruited, it is cut down to the ground, because each trunk produces only one bunch of fruit. The dead trunk is replaced by others in the form of suckers, or shoots, which arise from the rhizome at roughly six-month intervals. The life of a single rhizome continues for many years, and the weaker suckers that it sends up through the soil are periodically removed, while the stronger ones are allowed to grow into fruit-producing plants (<https://www.britannica.com/plant/banana-plant>).

1.2. Nutritional composition of banana

Bananas are a nutritious fruit in terms of their carbohydrate and sugar contents. A ripe fruit contains as much as 22% of carbohydrate, mainly as sugar, and is high in

dietary fibre, potassium, manganese, and vitamins B₆ and C. Almost all the modern edible parthenocarpic bananas come from the two wild species-*M. acuminata* and *M. balbisiana*. Other than fresh fruits, it can be consumed as processed in various forms like chips, powder, flakes, etc. Banana pseudo stem is chopped and used as cattle feed. A medium sized banana has about 105 calories. Table 1 provides the information on the nutritional value per 100 g of raw banana.

Table 1. Nutritional composition of 100 g of raw banana

S. No.	Nutrient	Quantity	S. No.	Nutrient	Quantity
1.	Carbohydrates	22.84 g	5.	Energy	89 kcal
	Sugars	2.23 g	6.	Minerals	
	Dietary fibre	2.6 g		Iron	0.26 mg
2.	Fat	0.33 g		Magnesium	27 mg
3.	Protein	1.09 g		Manganese	0.27 mg
4.	Vitamins			Phosphorus	22 mg
	Thiamine (B ₁)	0.031 mg		Potassium	358 mg
	Riboflavin (B ₂)	0.073 mg		Sodium	1 mg
	Niacin (B ₃)	0.665 mg		Zinc	0.15 mg
	Pantothenic acid (B ₅)	0.334 mg	7.	Water	74.91 g
	Vitamin B ₆	0.4 mg			
	Folate (B ₉)	20 µg			
	Choline	9.8 mg			
	Vitamin C	8.7 mg			

Source: USDA Nutrient Database (2018)

2 Banana Cultivation

In recent years, the recognition of the importance of bananas and plantains, as household food, nutritional security and social security, has grown in many parts of the world. Today bananas are cultivated in more than 150 countries with a total annual world production of 113.91 million tonnes (FAOSTAT, 2017). There are more than 1000 varieties of bananas produced and consumed locally in the world, but the most commercialized is the Cavendish type banana, which accounts for around 47 percent of global production. Cavendish banana crops are able to achieve high yields per hectare and due to their short stems, are less prone to damage from environmental influences such as storms. Cavendish banana plants are also known for recovering from natural disasters quickly. Approximately 50 billion tonnes of Cavendish bananas are produced globally every year (Banana Facts and Figures -FAO<http://www.fao.org/economic/est/estcommodities/bananas/bananafacts/en/#.XLVslkiYPbg>). Locally consumed bananas are a staple food in many tropical countries and play a major role in terms of contributing for food security. Major banana producing countries in the world are India, China, Angola, the Philippines, Brazil, Ecuador, Indonesia, Guatemala, Colombia and Cameroon. India is the top banana producing nation in the world contributing to 26.7% of the total world production. Table 2 presents the major banana producing countries in the world.

Table 2. Major banana producing countries in the world

Country	Production (Million tonnes)	Share (%) in total world production
India	30.47	26.7
China	11.42	10.02
Indonesia	7.16	6.2
Brazil	6.67	5.8
Ecuador	6.28	5.5
Philippines	6.04	5.3
Angola	4.30	3.7
Guatemala	3.88	3.4
Colombia	3.78	3.3
Cameroon	1.24	1.0
Other countries	32.67	28.6
World	113.91	

Source: FAOSTAT (2017)

Banana cultivation is one of the most popular agricultural practices in India as the fruit grows all-round the year irrespective of other fruits, which are usually seasonal in nature. It is the largest produced and maximally consumed amongst the fruits cultivated in India. It is grown in almost all part of the country. As per the All India Third Advance Estimates of Area & Production of Horticulture Crops, Ministry of Agriculture & Farmers Welfare, the total production of banana was 31.08 million tonnes from 8,59,000 ha cultivated area during 2017-18. About 17% of total cultivated banana area is under TC raised plants of Grand naine (G9). Major banana producing states in India are Gujarat, Andhra Pradesh, Tamil Nadu, Uttar Pradesh, Maharashtra, Karnataka, Madhya Pradesh, Bihar, Kerala, West Bengal, Assam, Chhattisgarh, Odisha. Gujarat leads in production (4.18 million tonnes) (Fig. 1) and Karnataka leads in area under cultivation (102,000 ha) (Fig. 2) (NHB, 2017).

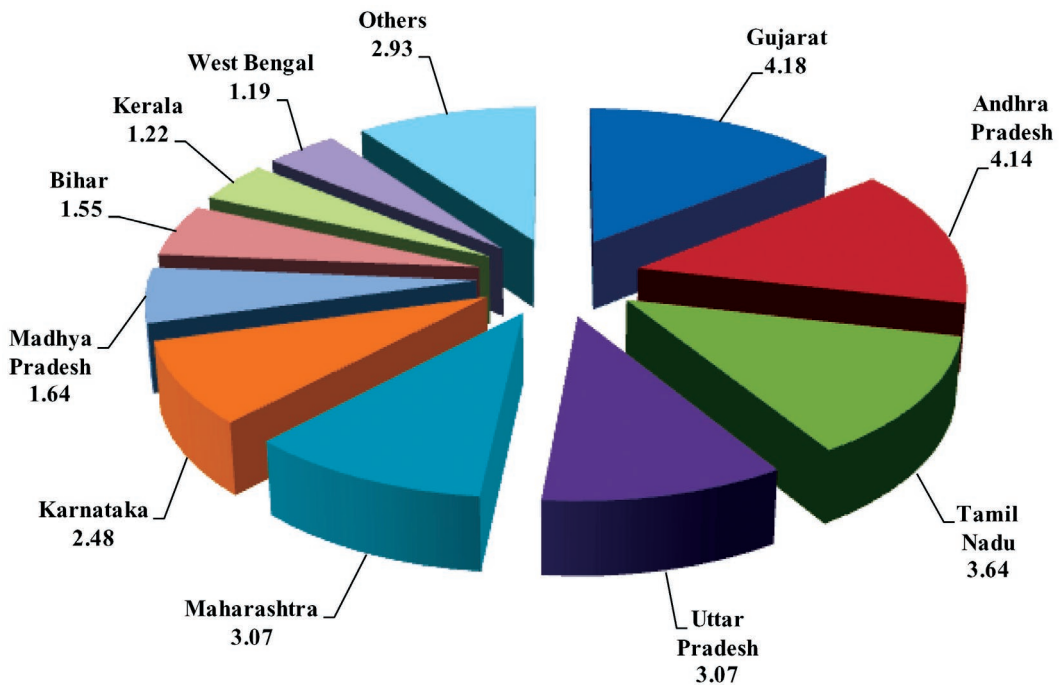


Fig. 1. Banana production (million tonnes) in different states of India in 2016-17 (NHB, 2017)

India is home to wide range of *Musa* cultivars with varying genomic status and diverse agro-climatic conditions, which has encouraged the development and sustenance of large number of varieties catering to local needs (NRCB, Vision 2050). Most of the bananas grown in India are for the domestic market. Even though more than half of the bananas produced in India are Cavendish cultivars, but the country is host to a wide diversity of bananas. More than 20 types of bananas are grown mainly in South India such as, Rasthali, Nendran, Karpuravalli, Kunnan, Ney Poovan, Thella Chakkara Keli and Monthan (Fig. 3). Of the large number of varieties cultivated, G9

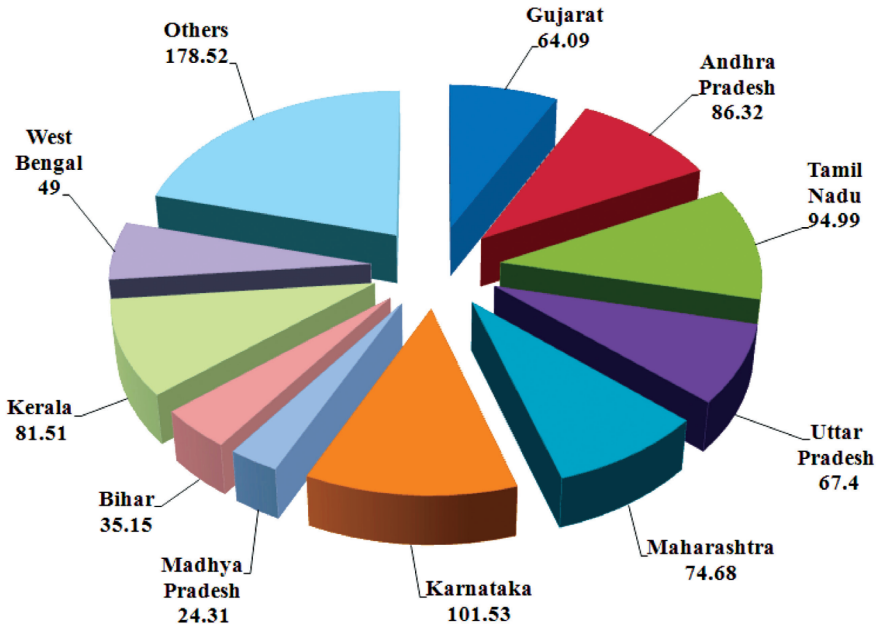


Fig. 2. Area under banana production ('000 Ha) in different states of India in 2016-17 (NHB, 2017)

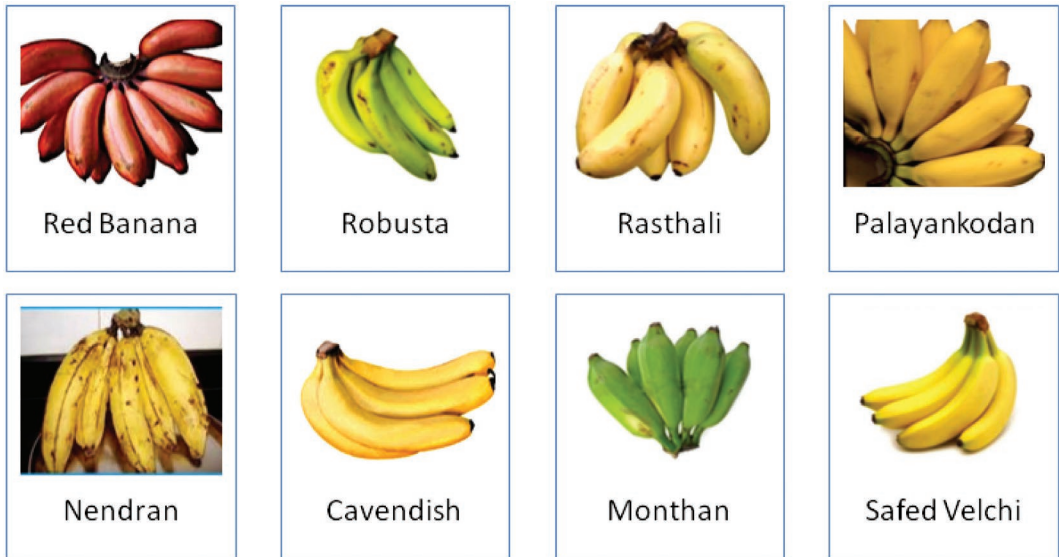


Fig. 3. Important banana varieties cultivated in India

is the most preferred variety for the farmers. It is a high yielding Cavendish variety brought to India from Israel during 1995 and adopted for commercial production. About 5 million TC plantlets of G9 are planted in about 17% of total cultivated banana area in India every year. It has shorter ratooning time, more productive and most important commercial clone worldwide due to its production of large bunches and fingers, delicious in taste, longer shelf life as compared to native varieties, and

attractive yellowish green color at maturity. It is internationally acceptable both as fresh fruit and in processed form due to high pulp peel ratio. Bananas are both grown in homestead gardens and cultivated commercially on large areas. Commercial production is largely dependent on irrigation whereas subsistence cultivation is practiced under rain-fed conditions. Important banana varieties cultivated in different states of India are given below in Table 3:

Table 3. Important banana varieties cultivated in different states of India

State	Major Varieties grown
Andhra Pradesh	Dwarf Cavendish, Robusta, Rasthali, Amritpant, Thellachakrakeli, Karpoora Poovan, Chakrakeli, Monthan and Yenagu Bontha
North Eastern States	Jahaji (Dwarf Cavendish), Chini Champa, Malbhog, Borjahaji (Robusta), Honda, Manjahaji, Chinia (Manohar), Kanchkol, Bhimkol, Jatikol, Digjowa, Kulpait, Bharat Moni, Sabri
Bihar	Dwarf Cavendish, Alpon, Chinia, Chini Champa, Malbhig, Muthia, Kothia, Gauria
Gujarat	Dwarf Cavendish, Lacatan, Harichal (Lokhandi), Gandevi Selection, Basrai, Robusta, G-9, Harichal, Shrimati
Jharkhand	Basrai, Singapuri
Karnataka	Dwarf Cavendish, Robusta, Rasthali, Poovan, Monthan, Elakkibale, Nanjangudrasabalehannu
Kerala	Nendran (Plantain), Palayankodan (Poovan), Rasthali, Monthan, Red Banana, Robusta
Madhya Pradesh	Basrai
Uttar Pradesh	Grand Naine, Dwarf Cavendish, Alpon, Chinia, ChiniChampa
Maharashtra	Dwarf Cavendish, Basrai, Robusta, Lal Velchi, Safed Velchi, Rajeli Nendran, Grand Naine, Shreemanti, Red Banana
Orissa	Dwarf Cavendish, Robusta, Champa, Patkapura (Rasthali)
Tamil Nadu	Virupakshi, Robusta, Rad Banana, Poovan, Rasthali, Nendran, Monthan, Karpuravalli, Sakkai, Peyan, Matti
West Bengal	Champa, Mortman, Dwarf Cavendish, Giant Governor, Kanthali, Singapuri

Source: http://nhb.gov.in/report_files/banana/BANANA.htm

3 Banana Cultivation Practices

Banana cultivation is a very profitable agriculture business in India. Banana is basically a tropical crop and having a tropical climate most of the year; it can be grown almost all round the year.

3.1. Climate and Soil

Banana grows well in a temperature range of 15°C-35°C with relative humidity of 75-85%. It can be successfully grown at an altitude of 1200 meters from sea level in a warm and humid climate. There is retardation in growth below 20°C and above 35°C. Banana takes longer time to mature in cooler climates while the growth and yield is reduced at lower humidity and temperature. Banana is a water loving plant and it requires a large quantity of water for maximum productivity. Four months of monsoon (June to September) with an average 650-750 mm rainfall are most important for vigorous vegetative growth of banana. Drought is a limiting factor in banana cultivation. Periods of drought or low temperature reduces growth and flowering with the result that the peak production period is delayed. Deep, rich loamy soil with pH in range of 6.5-7.5 is most preferred for banana cultivation. Soil for banana should have good drainage, adequate fertility and moisture. Saline solid, calcareous soils are not suitable for banana cultivation. A soil which is rich in organic material with high nitrogen content, has adequate phosphorus level, plenty of potash and is neither too acidic nor too alkaline, is good for banana(http://nhb.gov.in/report_files/banana/BANANA.htm).

3.2. Irrigation

Being a tropical crop, the water requirement of banana is very high, it needs an annual rainfall of 2000 to 4000 mm, evenly distributed over different parts of the year. For the entire life cycle, banana needs 900-1200 mm of water. This is generally met through rainfall and the extra requirement is provided through irrigation. The moisture level should be optimum during all growth stages and the excess water should be drained out from the root zone. In winter, irrigation is provided at an interval of 7-8 days while in summer, it should be given at an interval of 4-5 days. Drip irrigation, trench irrigation and flood are some of the common irrigation systems followed for cultivation of banana. Drip irrigation is the most economical and popular one as it ensures water being dispensed at the root zone (Fig. 4). It also helps in maintaining the proportion of soil air and soil water which results in early



Fig. 4. Tissue cultured banana (G9) cultivation using drip irrigation system (Photo by Nishant Srivastava)

and vigorous growth of bunches. Table 4 presents the drip water requirement at different growth stages of banana. The application of drip irrigation and mulching technology has reported improved water use efficiency. There is saving 56% of water and increase of yield by 23-32% under drip irrigation system.

Table 4. Drip irrigation requirement at different growth stages of banana

S. No.	Crop growth stage	Duration after planting (weeks)	Quantity of water (litre/plant)
1.	After planting/Ratoon	1-4	Flood irrigation
2.	Juvenile phase	5-9	8-10
3.	Critical growth stage	10-19	12
4.	Flower bud differentiation stage	20-32	16-20
5.	Shooting stage	33-37	20 and above
6.	Bunch development stage	38-50	20 and above 8

Source: National Research Centre for Banana, Tiruchirapalli, Tamil Nadu

3.3. Planting

Banana can be planted throughout the year except in severe winter and during heavy rains when the soil remains very wet. The ideal time (October-November)

of planting is after the monsoon season but with assured irrigation, the planting can also be done in February-March. Levelled or plain land is thoroughly ploughed before planting to break down the soil clods. Stones, rocks and other debris must be removed. On hill slopes, only individual pits are prepared for placing the planting material, instead of the whole area, to avoid soil erosion. Spacing is affected by the growth habits of the plant and the fertility of soil. The most common method of planting followed is pit planting. The pits are modified as per planting requirement using manure, gypsum and neem cake. The suckers are planted at the centre of the pit and the soil around it is packed tightly. Deep planting in banana cultivation must be avoided. The plants are irrigated 3-4 days before planting and immediately after planting. Trench planting is practiced along the Cauvery delta region, while furrow planting is done in annual planting system in Maharashtra and Gujarat. The time of pit preparation is linked with the time of planting. Generally, pits are dug 30-40 days before planting to allow the soil to get naturally sterilized. In case sufficient sunlight is not available, the open pits are sterilized by burning the fire wood. This practice also adds potassium in the pits. Plant population depends on cultivars, topography and soil fertility (Table 5).

Table 5. Plant population under different planting systems

S. No.	Method of Planting	Spacing (m)	Plant Population/ha
1.	CONVENTIONAL PLANTING		
i)	Dwarf Cavendish	1.5 × 1.5	4440
ii)	Robusta and Nendran	1.8 × 1.8	3080
iii)	Rasthali, Poovan, Karpuravalli, Monthan	2.1 × 2.1	2260
2.	HIGH DENSITY PLANTING		
a)	Paired row planting system		5200
i)	Dwarf Cavendish	1.2 × 1.2 × 2.0	3800
ii)	Robusta, Grand Naine, Poovan, Rasthali and Ney Poovan	1.5 × 1.5 × 2.0	
b)	3 suckers/hill (Robusta, Nendran)	1.8 × 3.6	4500

Source: National Research Centre for Banana, Tiruchirapalli, Tamil Nadu

3.4. Fertigation

There are three critical stages of growth recognized in banana viz., vegetative, floral and fruit development. The number and size of fruits ultimately borne by the shoot is determined by the vegetative growth in the first six months from planting. Better vegetative growth *i.e.* a large leaf area is important for a high yield. Proper growth and production of fruits can be achieved by judicious application of manures

and fertilizers. Banana requires high amount of nutrients, which are often partly supplied through the soil. The nutrient requirement on all India basis is worked out to be 20 kg FYM, 200 g N; 60-70 g P; 300 g K/plant. Banana crop requires 7-8 kg N, 0.7- 1.5 kg P and 17-20 kg K per metric tonne yield. In order to avoid loss of nutrients from conventional fertilizers *i.e.* loss of N through leaching, volatilization, evaporation and loss of P and K by fixation in the soil, application of water soluble or liquid fertilizers through drip irrigation (fertigation) is encouraged. A 25-30% increase in yield is observed using fertigation. Moreover, it saves labour and time and the distribution of nutrients is uniform.

Table 6. Weekly fertigation schedule for banana (g/plant/application)

Weeks after planting	Urea	Total (g/plant)	Muriate of potash (MOP)	Total (g/plant)
9 to 18 week (10 weeks)	15	150	8	80
19 to 30 week (12 weeks)	10	120	10	120
31 to 40 week (10 weeks)	7	70	12	120
41 to 46 week (5 weeks)	Nil	Nil	10	50
Total	–	340	–	375

Source: National Research Centre for Banana, Tiruchirapalli, Tamil Nadu

3.5. Special operations

There are operations specific to banana crop that help in increasing their productivity and quality.

- a. **Desuckering** - Desuckering is important operation in banana cultivation to reduce internal competition with mother plant. It involves the removal of unwanted suckers which develop near the base of the plant.
- b. **Propping** - It is an essential cultural practice to give proper support to the plants with bamboos. It is done to avoid falling down of plants due to high winds (Fig. 5).
- c. **Pruning** - Pruning of surplus leaves helps to reduce the disease from spreading through old leaves.
- d. **Earthing** - To avoid water logging, earthing up should be done at 3-4 months after planting *i.e.* raising the soil level around the base of the plant.
- e. **Weeding** - To keep the plantation weed free, it is important to spray Glyphosate (Round up) before planting at the rate of 2 lit/ha. One or two manual weedings are necessary.



Fig. 5. Propping of banana plants from tissue cultured G9 variety using bamboo (Photo by Shiv Kant Shukla)

- f. **Wrapping** - Banana bunch is covered to protect the fruits from sunburn, hot wind and dust. Wrapping is also done to improve the colour of the fruit. It also prevents insect bites and its resulting black spots. This practice helps in maintaining the good quality of banana fruit (Fig. 6).

3.6. Harvesting

Banana is harvested when the fruit is slightly or fully mature depending on the market preferences. For long distance transportation, harvesting is done at 75-80% maturity. The fruit is climacteric and can reach consumption stage after ripening operation. Harvesting is done within 12-15 months of planting and the main harvesting season of banana is



Fig. 6. Wrapping of banana fruit (Photo by Shiv Kant Shukla)

from September to April. Bunches attain maturity from 90-150 days after flowering depending upon variety, soil, weather condition and elevation. The banana bunches can be directly cut from the plants in fields and other operations like cutting the hands, application of fungicides, are carried out in the shade as sunlight is detrimental to the shelf life of bananas. The bunch must be green in color, 75% ripe, free from injuries and blemishes. Harvested bunch should generally be collected in well-padded tray or basket and brought to collection site. Bunches should be kept out of light after harvest, since this hastens ripening and softening.

4 Constraints in Banana Cultivation

In India, banana occupies 13% area out of total area under fruit crop in India (NHB, 2017). It is grown under diverse conditions and production systems. Most of the bananas are traditionally grown by planting suckers. Rhizomes and peepers are the other planting materials used for banana cultivation. There are two types of suckers *viz.*, sword sucker and water sucker (Fig. 7). Suckers that produce leaves with a midrib and a very narrow lamina are sword suckers. Water suckers usually have a small rhizome and broad leaves. However, fruits produced through water suckers are of inferior quality and hence not used in commercial farming. Sword suckers with well-developed rhizome, conical or spherical in shape having actively growing conical bud and weighing 450-700 g are commonly used as propagating material. In banana farming, suckers may be infected with some pathogens and nematodes. Due to variation in age and size of sucker, crop is not uniform, harvesting is prolonged and management becomes difficult. Further, the high cost of suckers, poor quality, lack of scientific knowledge about planting technique and unavailability of sufficient suckers at the time of planting are some of the limitations related to sucker planting technique. Biotic and abiotic stresses are the other main constraints which reduce the productivity considerably. The constraints in production of banana also vary from region to region.

Most of the above constraints can be addressed through the use of banana tissue cultured planting material.

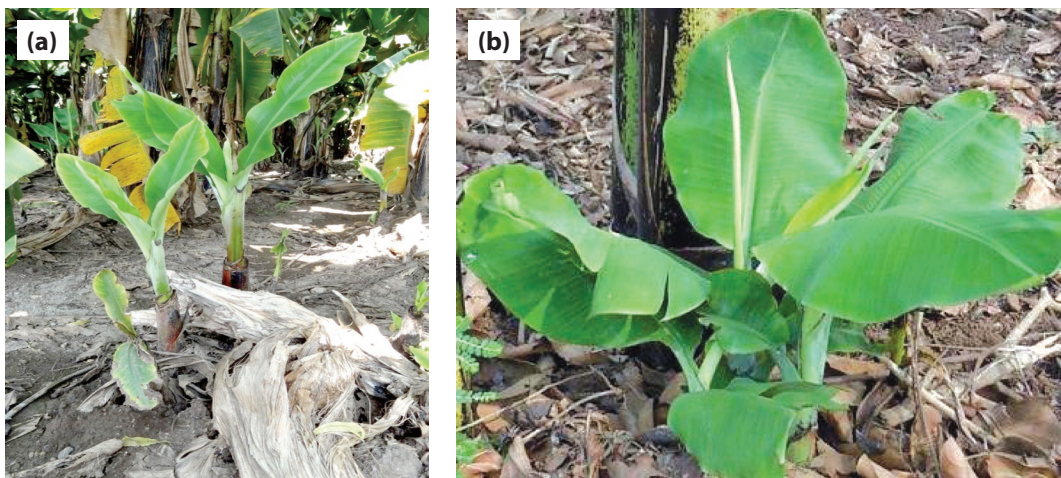


Fig. 7. (a) Sword sucker (Photo by Nishant Srivastava) and (b) water sucker (<http://www.promusa.org/Banana+sucker>)

5 Banana Tissue Culture in India

In India, banana cultivation through tissue culture (TC) is the recent trend relatively. Tissue culture is the propagation of a plant part or single cell or group cell in a test tube under controlled and aseptic conditions. It has laid the foundation of production of uniform, high quality, disease free planting material and true-to-type plants at a mass scale. About 70% farmers in India are using suckers while the remaining 30% of the farmers are using TC banana plants (http://nhb.gov.in/report_files/banana/BANANA.htm). Tissue culture banana production technology is superior over conventional sucker propagation with respect to the following advantages:

- True-to-type to the mother plant under aseptic conditions.
- Pest- and disease-free seedlings.
- Uniform growth of crop with increase in yield.
- Early maturity of crop - maximum land use is possible by smallholder farmers in country like India.
- Round the year planting possible as seedlings are made available throughout the year.
- Two successive ratoons are possible in a short duration which minimizes cost of cultivation.
- No staggered harvesting is required.
- About 95%-98% plants bear bunches.
- New varieties can be introduced and multiplied in a short duration.

The first commercial micropropagation laboratory in India was set up in 1987 in Cochin Export Processing Zone under the name A.V. Thomas & Co. It was followed by Indo-American Hybrid Seeds, Bangalore; Hindustan Lever Ltd., Mumbai and Unicorn Biotek, Hyderabad, in 1988. The Jain Tissue Culture laboratory in Jalgaon district of Maharashtra pioneered the tissue culture of G9 variety of banana since 1994-95. It is the largest banana TC laboratory in the country with a production capacity of 85 million plants per annum. In 2018, 38.57 million banana TC plants were produced and certified by the Jain Irrigation Systems. Rise and Shine Biotech, in Pune, Maharashtra, is another major TCPF that produced 28.8 million banana TC plants in 2018.

Banana is the major cultivated TC-raised crop in India. The installed production capacity in India to produce TC-raised planting material is 650 million plantlets per annum and actual supply is 455 million plantlets per annum. Annual requirement of suckers/planting material of banana in India is 3436 million, which shows there is huge potential to produce TC-raised planting material to meet the current demands of farmers.

5.1. Protocols for banana tissue culture

The tissue culture protocol for banana is very well standardized in India. The standard guidelines for the production of TC raised banana are described in detail at <http://dbtncstcp.nic.in/Portals/0/Images/BANANA.pdf>. The basic steps involved in the production of tissue culture banana (TCB) are as follows:

a. Selection of a superior mother clone

The selected mother plants should be healthy and free from pests and diseases. Each and every mother plant should be screened especially for viruses before planting in mother nursery and maintained in virus-free greenhouse until used in tissue culture production.

b. Initiation of culture

The ex-plants for initiation are taken from mother plants that have been tested and maintained in virus-free conditions. The ex-plants are thoroughly sterilized, excised and planted on the culture media as per the standard operating procedures. Valid virus testing is required at the 2nd /3rd subculture stage prior to the bulking up of the cultures.

c. Multiplication

In order to minimize the chances of soma clonal variations, shoots of banana should not be subcultured for more than 8th cycle. Shoot multiplication process in banana is strictly monitored to maintain clonal uniformity of the progeny.

d. Rooting

The individual shoots are transferred to the rooting media and once the roots are formed, the tissue-cultured banana plantlets are hardened in a partially shaded polyhouse/greenhouse and further acclimatized in an outdoor nursery.

e. Dispatch

Prior to dispatch to the farmers, the tissue-cultured plants growing in the nursery should be tested for the absence of the viruses infecting banana and clonal uniformity. Plants should have label of certification from accredited test laboratories.

6 Journey of Certification of Banana in India

Tissue culture banana is very popular among growers and widely accepted in India. Majority of TC companies are engaged in mass multiplication of banana. It covers almost 70% of total production of TC plants (Shukla, 2016). Tissue culture plants offer various advantages over the conventional planting materials (suckers) of banana in terms of better vegetative growth and yield. However, the TC propagation does not exclude the infection of viruses unless the mother plant material used for TC production and the subsequent stock-cultures are tested and maintained free from viruses. It is, therefore, essential that proper testing of above material is done for purpose of certification with respect to its virus-free status.

To promote TC industry in India, the Department of Biotechnology (DBT) established a network project on National Facility for Virus Testing & Quality Control in 1999. Subsequently in March 2006, the National Certification System for Tissue Culture Raised Plants (NCS-TCP) was established by DBT, Ministry of Science & Technology, Government of India, for the first time to provide support to plant tissue culture industry to facilitate production of quality planting material through tissue culture/micropropagation. Biotech Consortium India Limited (BCIL) on behalf of DBT has been playing a vital role in implementation of NCS-TCP in India (Shukla, 2017). Currently, 82 TC companies are recognized under NCS-TCP. The incidence of virus infection has significantly reduced and no major virus outbreaks have been reported during the last few years since the implementation of the certification programme. Under NCS-TCP, approximately 28 million TC banana plants produced by the Indian tissue culture companies have been certified in 2016-17. The certification of plants ensures that only good quality, disease-free and true-to-type plants are being supplied to the farmers which eventually helps in their growth and sustainability. Fig. 8 shows the location of TC companies involved in banana production in India. These companies produce 5 million to 75 million of banana plantlets depending upon their capacity.

Quality certification of banana under NCS-TCP includes two main aspects:

a. Virus Indexing

In India, there are four major viruses known to naturally infect banana *viz.*, banana bunchy top virus (BBTV), banana streak Mysore virus (BSMYV), banana bract mosaic virus (BBrMV) and cucumber mosaic virus (CMV). Fig. 9 shows banana plants

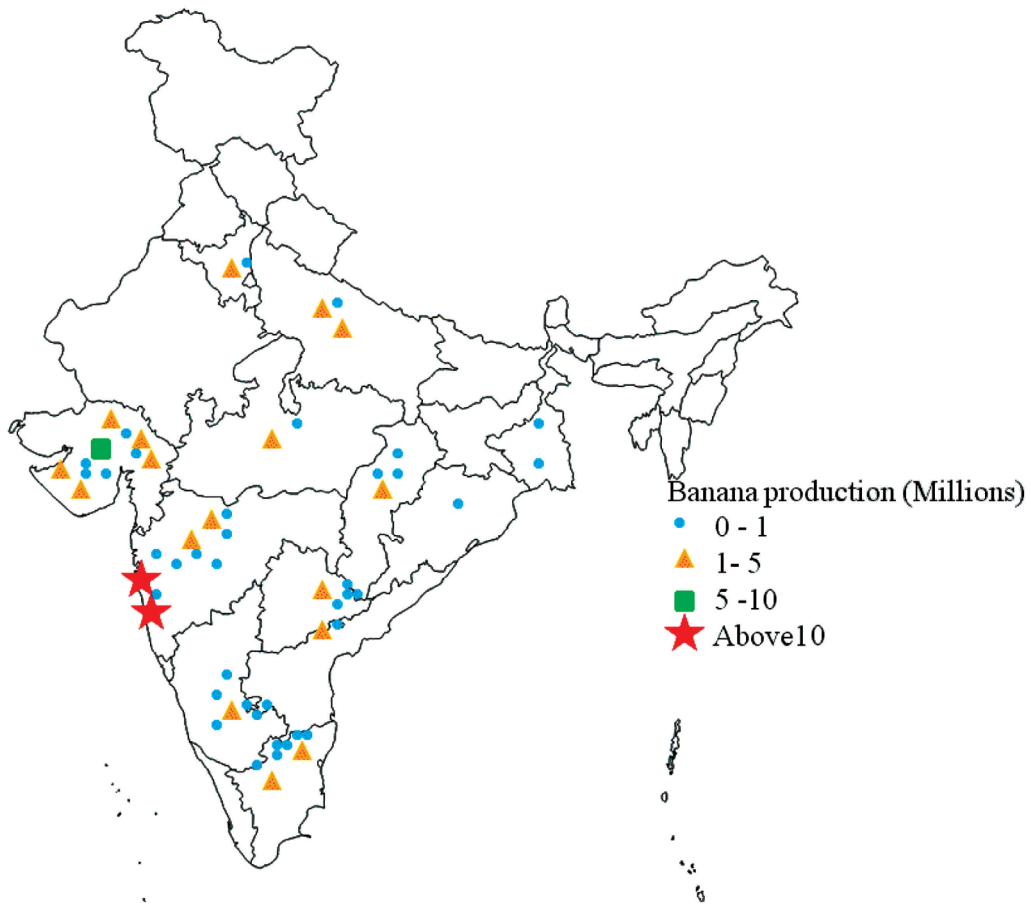


Fig. 8. Location of banana tissue culture companies recognized under NCS-TCP

with symptoms of infection with different viruses. BBTv, BSMYV are the DNA viruses and polymerase chain reaction (PCR) based tests are carried out for their detection. CMV, BBrMV are the RNA viruses infecting banana and are tested by enzyme-linked immunosorbent assay (ELISA).

b. Genetic fidelity

Uniformity in TC planting material in terms of homogeneity is important in order to plan the production cycle of the crop by the farmers. Somaclonal variations are considered as a major factor for inducing genetic variability in the TC plants, thus, affecting its yield in the farmers' fields. Sometimes variants continue to multiply in the form of *in vitro* culture which leads to production of variant population within the batch. Such variants need to be detected in TC plants before supply to farmers through PCR based analysis. Various molecular markers such as Inter Simple Sequence Repeats (ISSR), Inter Retroposon Amplified Polymorphisms (IRAP) are being used for genetic fidelity testing.

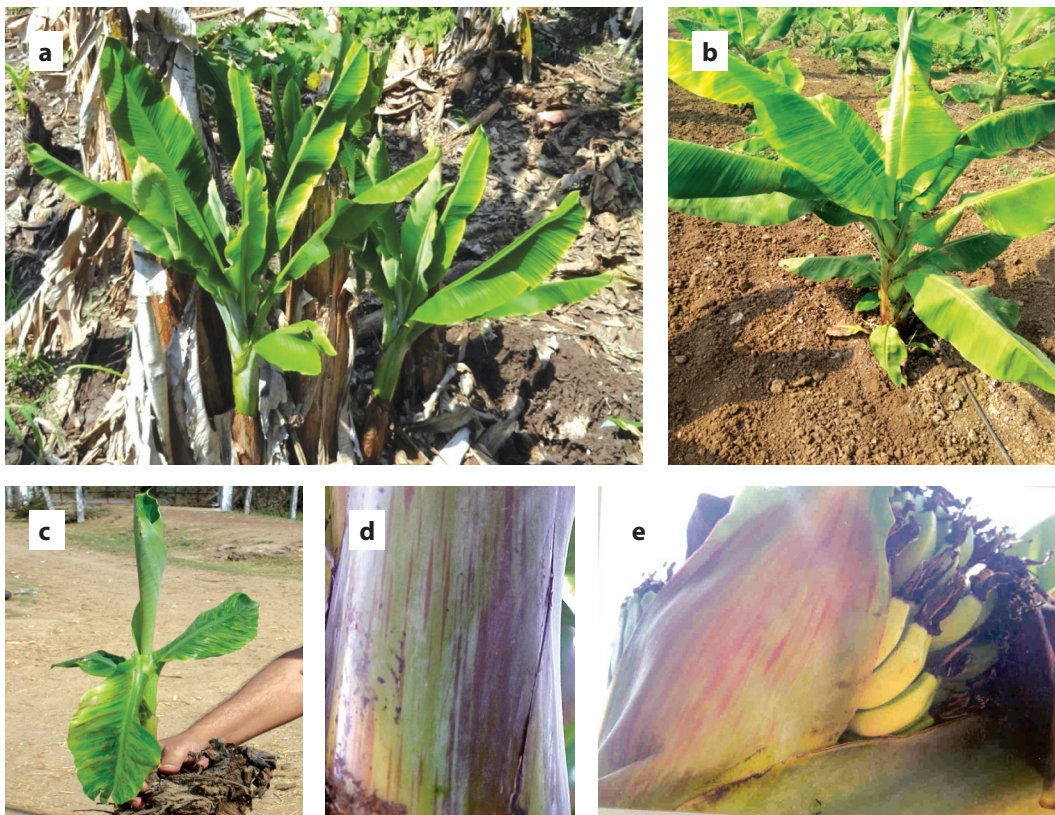


Fig. 9. (a) Banana plant with bunchy top symptoms caused by banana bunchy top virus (b) Yellow streaks symptoms on banana leaf caused by cucumber mosaic virus (a & b) Photo by Nishant Srivastava (c) banana leaf showing chlorotic streaks caused by banana streak virus (d) banana stem and flower (e) affected with banana bract mosaic virus (c, d & e) Photo by V.K. Baranwal

6.1. Steps involved in the certification of tissue culture banana

The various steps involved in the certification of TCB have been described below (Shukla, 2016):

a. Recognition under NCS-TCP

The first step for entering into plant certification involves getting the commercial Tissue Culture Production Facility (TCPF) recognized under the NCS-TCP. The recognition of TCPFs is based on the compliance with defined parameters broadly categorized into infrastructure, effective supervision of production process, package of practices for the crop which is being multiplied at commercial scale using tissue culture, maintenance of records and documents.

b. Indexing of stock culture/mother plant tissue

Under NCS-TCP, indexing the mother plants and the subsequent batch culture for all the four banana viruses is necessary. The recognized TCPF need to get all

the mother plants tested from the Accredited Test Laboratory (ATL) prior to mass multiplication at commercial level. The respective batch of TC plant should be derived from the tested mother culture. The ATL assigns a unique 20 digit batch number to the original test material.

c. Restricting the multiplication cycle

In order to ensure genetic uniformity and prevent somaclonal variation, the *in vitro* multiplication in case of TC banana should be restricted to the eight cycles. After eight cycles, all the multiple shoots should be transferred for rooting and thereafter the rooted plants should be sent for hardening in the greenhouse/polyhouse (Fig. 10).



d. Maintenance of records/unique code and batch number

The maintenance of batch is the basic requirement for certifying plants and to ensure

traceability. The ATL assigns a unique 20 digit batch number to original test material and after testing of viruses test report is prepared. Each activity (from selection of mother plants up to dispatch of hardened plants for planting) should be documented systematically.

Fig. 10. Hardened banana plants in the net house (Photo by V.K. Baranwal)

e. Certification of plants (before dispatching to farmers)

Prior to dispatch to the farmers, the TC plants being hardened/acclimatized in the insect proof greenhouse and net house, are drawn for virus indexing and testing of genetic fidelity. The sample size should be 1% for the batch of 1000 plants, 0.5% for the batch of plants ranging from 1001-10000 and 0.1% for the batch consisting of 10001-1,00,000 plants. The ATLs assign additional 20 digits batch registration number and the ATL issues certificate of qualities along with certification labels.

7 Accreditation of Labs

The Tissue Culture Certification Agency (TCCA) has identified two Referral Centres (RCs) and four ATLs under NCS-TCP to cater to demand of testing and certification of recognized TCPFs.

7.1. Referral Centres

- Referral Centre for Virus Indexing- Indian Agricultural Research Institute (IARI), New-Delhi
- Referral Centre for Genetic Fidelity/Uniformity - National Institute on Plant Biotechnology (NIPB), New-Delhi

The Referral Centres (RCs) are responsible for carrying out confirmatory test in the event of dispute or non-conformity of test results, developing, standardizing and validating test protocols for virus diagnosis, maintaining test records, conducting training for technical personnel working at ATLs and evaluating diagnostic reagents provided to ATLs. The training conducted is specifically aimed at increasing the proficiency of technical personnel working at ATLs for ELISA and PCR based tests for virus indexing along with PCR based tests for genetic fidelity. The RCs are not involved in the routine virus diagnosis/genetic fidelity/uniformity testing of TC plants. The RCs are responsible for validation of test result of 5% of samples sent by the respective ATLs.

7.2. Accredited Test Laboratories

- University of Agricultural Science (UAS), Bangalore
- Vasantdada Sugar Institute (VSI), Pune
- Indian Institute of Sugarcane Research (IISR), Lucknow
- Central Potato Research Institute (CPRI), Shimla

The ATLs are responsible for performing tests for both virus indexing and genetic fidelity. Based on the tests conducted in conformity with the standard protocol, the ATLs prepare a test report. On the basis of the test report, each ATL is authorized to issue the Certificate of Quality for the Tissue Culture Plant (CQ-TCP) along with certification label on behalf of the TCCA.

8 Indexing of TC Plants

Indexing of the mother plants for freedom from viral, bacterial, and fungal diseases is essential before undertaking large-scale plant propagation through tissue culture. The health status of the donor mother plant and multiplied TC plants is one of the most critical factors, which determine the success of a tissue culture operation. The insect pests like weevils and nematodes, pathogens like fungal and bacterial origin are eliminated through shoot tip culture but viral pathogens are not removed, hence, there is a need to ensure that all the mother plants used for tissue culture are free from diseases. Virus indexing or testing the plants for the presence or absence of transmissible viruses is important. Every meristem tip must be tested before using it as a mother plant to produce virus-free stock. The four major viruses naturally infecting banana *viz.*, BBTV, BSMYV, BBrMV and CMV can propagate easily through mother clones of banana and, therefore, sensitive and accurate detection of these viruses is very critical to ensure virus-free planting material and prevention of horizontal transmission by vectors to realize full potential of banana production. Timely and early detection of viruses can save the crop. The mother plants are indexed and assured to be free of viruses before micropropagation and the resulting TC plants are indexed again before dispatch to the farmers. Virus indexing through standard serological and molecular based tests has a positive impact on banana tissue culture production.

9 Protocols for Virus Indexing

Immuno- and nucleo-based techniques are mainly used for virus indexing. Immuno techniques in the form of enzyme-linked immunosorbent assay (ELISA) are based upon the specific recognition of viral antigens by antibodies. Polymerase chain reaction (PCR) based on *in vitro* amplification of DNA is the most widely used nucleo-based method for detection of plant viruses. Under NCS-TCP, double antibody sandwich enzyme linked immunosorbent assay (DAS-ELISA) is used for the detection of RNA viruses, CMV as well as BBrMV and duplex PCR for simultaneous detection of the DNA viruses BBTV and BSMYV (Table 7) (Baranwal *et al.*, 2015).

Table 7. Indexing techniques used for banana virus detection under NCS-TCP

Viruses	Technique		
	DAS-ELISA	PCR	RT-PCR
BBTV		✓	
BSMYV		✓ *	✓ **
CMV	✓		
BBrMV	✓		

*BSMYV test by PCR only for G9 (AAA) variety; **BSMYV test by RT-PCR for varieties other than G9

9.1. Double antibody sandwich (DAS-ELISA) for detection of CMV and BBrMV

DAS-ELISA (direct procedures) has been the most popular ELISA procedure for plant virus detection. It is a 4-step procedure: (i) coating the microtitre plate wells with antigen specific coating antibody (IgG); (ii) addition of antigen containing sample (A); (iv) addition of enzyme - labelled antibody (E); (iv) addition of enzyme substrate (S) and determination of enzyme - mediated colour reaction (Fig. 11).

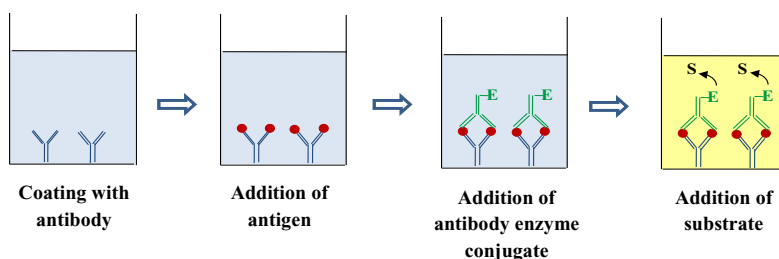


Fig. 11. Basic steps involved in DAS-ELISA

9.1.1. Protocol for DAS-ELISA

- Dilute virus specific coating antibody in coating buffer as recommended by the manufacturer and dispense 100 µl of the diluted antibody to the required number of wells (as per ELISA Plan). Cover the plate with aluminium foil and incubate at 37°C for 4 h or 4°C overnight.
- Decant and wash the plate by flooding the wells with PBS-T for about 3 min. Repeat wash and soak operations thrice and drain out residual liquid on a paper towel.
- Weigh test samples and grind using autoclaved mortar and pestle in extraction buffer at a ratio of 1:10 (w/v) at room temperature. Centrifuge at 12,000 rpm for 2 min and separate supernatant.
- Dispense 100 µl extract from test as well as healthy samples to each well of the microtitre plate. Cover the plate and incubate at 4°C overnight.
- Decant and wash the plate thrice as described above.
- Dilute specific antibody enzyme conjugates (as recommended by manufacturer) in PBS-TPO buffer and dispense 100 µl to each well and incubate at 37°C for 4 h.
- Decant and wash the plate thrice as described above.
- Dispense 100 µl freshly prepared substrate (p-nitrophenyl phosphate-PNPP, Sigma, USA) solution in substrate buffer (5 mg PNPP tablet in 10 ml substrate buffer) to each well. Incubate at 37°C for 1 h.
- Measure the intensity of color in each well at 405 nm by using ELISA reader after 30 mins and 1 h.
- Compare the absorbance values of the test samples with healthy control. Consider samples showing absorbance (OD_{405}) values more than two times of healthy control as positive (Fig. 12).

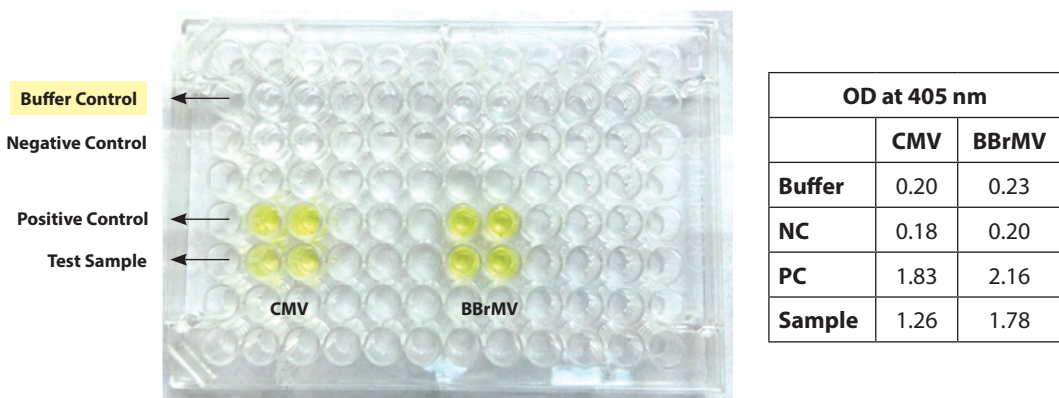


Fig. 12. DAS-ELISA analysis for cucumber mosaic virus & banana bract mosaic virus after 1 h of PNPP substrate addition. Healthy leaf of banana was used as negative control. CMV and BBrMV infected banana leaves were used as positive control (Source: Referral Centre for Virus Indexing, IARI, New Delhi)

9.2. Duplex PCR for detection of BSMYV and BBTV

PCR is a primer-mediated *in vitro* reaction involving amplification of target nucleic acid sequences (Mullis *et al.*, 1986). A standard PCR is a 3-step procedure: (i) denaturation at a high temperature (90°-95°C), (ii) annealing of target specific primers and (iii) primer extension by a thermostable DNA polymerase. Duplex PCR is the amplification of two target sequences in one reaction. A primer for detection is specific for each target sequence. Duplex PCR consists of two primer sets with same annealing temperature within a single PCR mixture to produce amplicons of varying sizes that are specific to different DNA sequences. The duplex PCR protocol for detection of BSMYV and BBTV has been described hereunder.

9.2.1. DNA extraction

Obtaining high quality intact DNA is the first and the critical step in performing PCR. Isolate DNA from test sample. The procedure mentioned below is for plant DNA isolation using DNeasy Plant Mini kit (Qiagen).

1. Weigh 100 mg test sample (leaf tissue) and disrupt using liquid nitrogen in an autoclaved pestle and mortar.
2. Soak the powdered sample immediately with 400 µl buffer AP1 and 4 µl RNase A. Vortex for 10 s and incubate at 65°C for 10 min in water bath. Invert the tubes 2-3 times during incubation.
3. Add 130 µl buffer P3. Mix and incubate for 5 min on ice followed by centrifugation at 20,000 g for 5 min at RT.
4. Transfer the clear lysate into a QIA shredder column and centrifuge for 2 min at 20,000 g.
5. Transfer the flow through to a new 1.5 ml Eppendorf tube and add 1.5 volumes of buffer AW1 and mix by pipetting.
6. Transfer 650 µl of the mixture to a DNeasy Mini spin column and centrifuge at 6000 g for 1 min. Discard flow through and repeat the step with remaining sample.
7. Place the spin column into a new 2 ml collection tube and add 500 µl buffer AW2 and centrifuge for 1 min at 6000 g.
8. Discard the flow through and add another 500 µl buffer AW2 and centrifuge for 2 min at 20,000 g.
9. Transfer the spin column to a new Eppendorf and add 30 µl buffer AE for elution. Incubate for 5 min at room temperature and centrifuge for 1 min at 6000 g.
10. Repeat the above step and store DNA at -20°C until further use.

9.2.2. Estimation of quality and quantity of DNA

Check the integrity of DNA by agarose gel electrophoresis and determine the quantity of DNA using Nanodrop spectrophotometer. A ratio of absorbance of 1.8 at 260 nm to that of 280 nm indicates that the sample is free from protein contamination. Good quality DNA usually have an A_{260}/A_{280} ratio of 1.7-1.8 and appear as an intact band on agarose gel.

9.2.3. Duplex polymerase chain reaction

Assemble the PCR reaction components on wet ice and prepare amplification mix by dispensing into 200 µl microfuge tube in the order listed below [See box].

Recipe of reaction mixture	
Reagents	Volume (ul)
10x Enzyme buffer	2.0
dNTP Mixture (10 mM)	1.0
BSV Mys F1 (10 µM)	1.0
BSV Mys R1 (10 µM)	1.0
BBTV F (10 µM)	1.0
BBTV R (10 µM)	1.0
AGMI 025 (10 µM)	1.0
AGMI 026 (10 µM)	1.0
DNA (100 ng/µl)	1.0
Taq DNA Polymerase (5 U/µl)	0.25
Sterile distilled water	9.75
Total volume	20.0

Primers used for detection of BSMYV and BBTV using duplex-PCR

Primer	Specific detection	Amplicon size	Reference
BSVMysF1	BSMYV RT/RNAse H region	~589 bp	Geering <i>et al.</i> (2000)
BSVMysR1			
BBTV F	Partial rep gene	~439 bp	Selvarajan <i>et al.</i> (2011)
BBTV R			
AGMI 025	STMS	~248 bp	Lagoda <i>et al.</i> (1998)
AGMI 026			

Note: Primers AGMI025 and AGMI026 are specific to *Musa* genomic DNA and used as internal control to detect amplification from *Musa* genome.

Mix the PCR reaction mixture well by inversion and place the tubes in a thermal cycler with the following reaction conditions [See Box].

PCR Temperature profile			
Steps	Temperature	Time	Cycle
Initial Denaturation	94°C	4 min	1
Denaturation	94°C	30 sec	35
Annealing	58°C	30 sec	
Primer extension	72°C	1 min	
Final primer extension	72°C	10 min	1

9.2.4. Electrophoresis analysis of amplicons

- Analyse the PCR products in 1.5% agarose gel in Tris-acetate EDTA (TAE) buffer containing ethidium bromide (0.5 µg/ml) (Fig. 13).

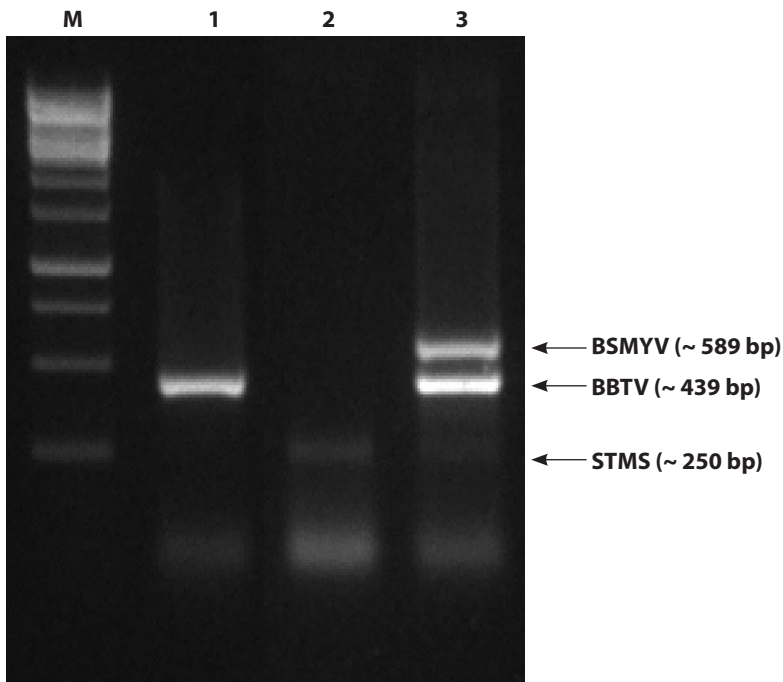


Fig. 13. Electrophoresis analysis of Duplex PCR. Lane 1: Test sample (~439 bp amplified BBTV DNA); Lane 2: Healthy control (~250 bp amplified fragment corresponding to Musa genome DNA); Lane 3: Positive control (~589 bp amplified BSMYV DNA, ~439 bp amplified BBTV DNA & ~250 bp amplified Musa genome specific DNA); M = 1 kb DNA ladder (Source: Referral Centre for Virus Indexing, IARI, New Delhi)

2. Melt 0.75 g agarose in 50 ml (1%) 1X TAE running buffer and add ethidium bromide (2.5 μ l) to the agarose after cooling to around 50°C.
3. Pour the melted agarose into the casting tray for polymerization and fill the buffer tank with running buffer (1X TAE) and remove the comb from polymerized agarose gel.
4. Load 25 μ l PCR product with 3 μ l 6X loading dye and run the gel at 60 V for 1 h along with 1 kb DNA marker (Thermo Scientific, India).
5. Examine the gel under ultraviolet transilluminator and take photograph.

9.3. Reverse transcription – PCR for detection of episomal BSMYV

Reverse transcription-PCR (RT-PCR) is the most commonly used method to detect RNA plant viruses using the enzyme reverse transcriptase. Reverse transcriptases (RTs) use an RNA template and a short primer complementary to the 3' end of the RNA to direct the synthesis of the first strand cDNA, which can be used directly as a template for the Polymerase Chain Reaction (PCR). BSV exists in either the infectious episomal form with a circular double stranded DNA genome, or the endogenous form in which the viral genome is integrated into the banana genome. PCR often leads to false positives due to amplification of integrated virus sequences. The integrated sequences are present in both *M. acuminata* (A) and *M. balbisiana* (B) genome, but the activable endogenous viral genome has been detected only in the B genome which are expressed in hybrids. Hence, for G9 variety (AAA), PCR can safely be used for BSMYV indexing but for other varieties (AAB, ABB), RT-PCR is employed for targeting the transcripts of episomal BSMYV. A standard RT-PCR is a 4-step procedure: (i) cDNA synthesis using reverse transcriptase at 42°C; (ii) denaturation at a high temperature (90°-95°C); (iii) annealing of target specific primers and (iv) primer extension by a thermostable DNA polymerase.

9.3.1. RNA extraction

Isolate RNA from infected leaf sample. The procedure mentioned below is for plant RNA isolation using RNeasy Plant Mini kit (Qiagen).

1. Weigh 100 mg test sample (leaf tissue) and disrupt using liquid nitrogen in an autoclaved pestle and mortar.
2. Add 450 μ l buffer RLT or buffer RLC and 5 μ l β -mercaptoethanol to the powdered sample and transfer the lysate to a clean microcentrifuge tube. Incubate at 56°C for 3 min for proper cell lysis and vortex vigorously during incubation.
3. Transfer the lysate to a QIA shredder spin column (lilac) placed in a 2 ml collection tube. Centrifuge for 2 min at full speed at room temperature. Transfer

the supernatant of the flow-through to a new microcentrifuge tube without disturbing the cell-debris pellet.

4. Add 0.5 volume of ethanol (96-100%) to the cleared lysate and mix immediately by pipetting.
5. Transfer the sample (usually 650 μ l) with any precipitate to the RNeasy mini spin column (pink) in a 2 ml collection tube. Close the lid and centrifuge for 30s at 10,000 rpm. Discard the flow-through.
6. Add 700 μ l buffer RW1 to the RNeasy spin column. Close the lid and centrifuge for 30s at 10,000 rpm. Discard the flow-through.
7. Add 500 μ l buffer RPE to the RNeasy spin column. Close the lid and centrifuge for 30s at 10,000 rpm. Discard the flow-through.
8. Add 500 μ l buffer RPE to the RNeasy spin column. Close the lid and centrifuge for 2 min at 10,000 rpm. Discard the flow-through.
9. Place the RNeasy spin column in a new 2 ml collection tube. Centrifuge at 14000 rpm for 1 min to dry the membrane.
10. Place the RNeasy spin column in a new 1.5 ml collection tube. Add 30-50 μ l RNase free water directly to the spin column membrane. Close the lid and incubate for 1 min at room temperature.
11. Centrifuge at 10,000 rpm for 1 min to elute RNA and store it at -80°C until further use.

9.3.2. Estimation of quality and quantity of RNA

Determine the integrity of RNA using Nanodrop spectrophotometer. RNA has its absorption maximum at 260 nm and the ratio of the absorbance at 260 nm and 280 nm is used to assess the RNA purity of an RNA preparation. Pure RNA has an A_{260}/A_{280} of 2.1. Usually, a value of 1.8-2.0 indicates purity level of RNA.

9.3.3. Reverse transcription polymerase chain reaction

RT-PCR can be performed in 2 steps, first with c-DNA synthesis using RT enzyme followed by PCR amplification using DNA polymerase in separate microfuge tube.

(a) Reverse transcription

Assemble the RT reaction components on wet ice and prepare mix by dispensing into 200 μ l microfuge tube in the order listed below [See box].

Mix RT reaction assembly by inversion and place the tubes in a thermal cycler with following reaction conditions [See box].

Recipe of reaction mixture	
Reagents	Volume (µl)
5X Enzyme buffer	5.0
MgCl ₂ (25 mM)	3.0
dNTP mixture (10 mM)	1.25
BSV Mys R1 (10 µM)	1.0
Template (~500 ng)	5.0
Reverse transcriptase	0.5
Sterile distilled water	8.25
Total volume	25.0

Note: Prior to use RNA as a template, denature RNA (5 µl) along with 1 µl of reverse primer at 65°C for 5 min and then snap chill on ice for 5 min and use it as template for RT-PCR.

RT Temperature profile			
Steps	Temperature	Time	Cycle
Annealing	25.0	5 min	1
c-DNA synthesis	42.0	60 min	1
Inactivation	70.0	15 min	1

Note: The profile mentioned above is for Improm-II Reverse transcriptase (Promega). Parameters may vary if using enzyme from a different source.

(b) Polymerase chain reaction

Assemble the PCR reaction components on wet ice and prepare amplification mix by dispensing into a separate 200 µl microfuge tube in the order listed below [See box].

Recipe of reaction mixture	
Reagents	Volume (µl)
10X Enzyme buffer	2.0
dNTP mixture (10 mM)	1.0
BSV Mys F1 (10 µM)	1.0
BSV Mys R1 (10 µM)	1.0
c-DNA template	4.0
Taq DNA polymerase (5 U/µl)	0.25
Sterile distilled water	10.75
Total volume	20.0

Mix the PCR reaction mixture well by inversion and place the tubes in a thermal cycler with the following reaction conditions [See Box].

PCR Temperature profile			
Steps	Temperature	Time	Cycle
Initial Denaturation	94°C	4 min	1
Denaturation	94°C	30 sec	35
Annealing	58°C	30 sec	
Primer extension	72°C	1 min	
Final primer extension	72°C	10 min	1

9.3.4. Electrophoresis analysis of amplicons

Analyze the RT-PCR products in 1% agarose gel in Tris-acetate EDTA (TAE) buffer containing ethidium bromide (0.5 µg/ml) as described above. Fig. 14a shows duplex PCR analysis of TC Elakki banana variety, indicating positive amplification for BSMYV (~589 bp) in all the 10 samples. RT-PCR analysis further confirms the absence of episomal BSMYV in those 10 samples (Fig. 14b). The false positive reaction indicated by PCR was due to amplification of integrated virus sequence found in banana hybrids.

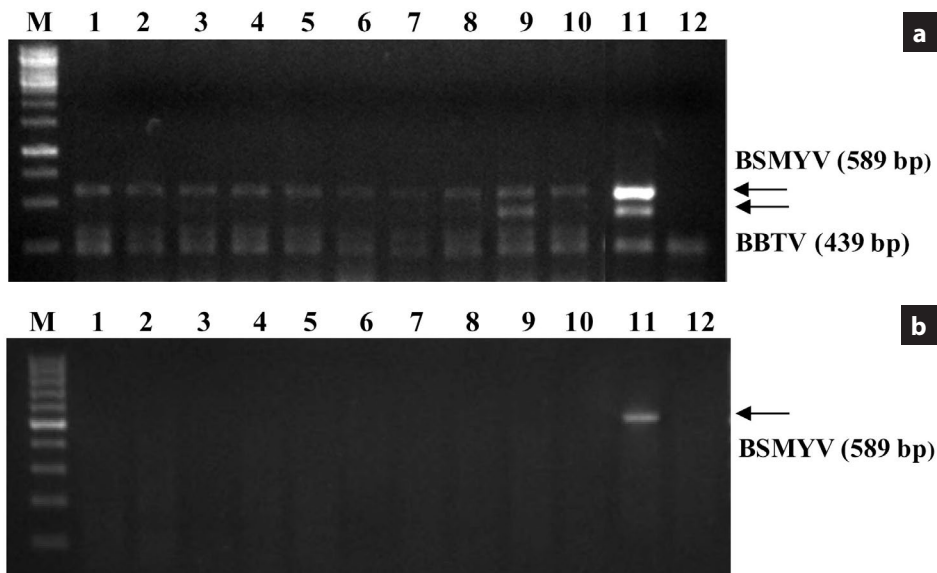


Fig. 14. (a) Duplex PCR of TC banana Elakki variety showing positive amplification of BSMYV and **(b)** Standard RT-PCR of TC banana Elakki variety showing no amplification for BSMYV. Lane 1-10: Test samples; Lane 11: Positive control; Lane 12: Negative banana healthy control. M = 1 kb DNA ladder (Source: Referral Centre for Virus Indexing, IARI, New Delhi)

9.4. Rapid and simplified virus indexing in resource constrained laboratories

Currently under NCS-TCP, banana virus indexing involves the use of ELISA and PCR-based techniques which are laborious, time consuming and require well equipped laboratories. With the advancement in molecular biology, various simple isothermal amplification techniques have been developed that can be easily performed with limited resources. One such isothermal technique that has been developed and widely used for detection of animal/plant viruses is recombinase polymerase amplification (RPA) (Pipenburg *et al.*, 2006; Daher *et al.*, 2016; Lobato *et al.*, 2018). In comparison to current PCR-based methods that rely on sophisticated equipments and complex procedures, RPA is simple, rapid, employs a single primer pair and can be performed at a constant low temperature (37°C-42°C) dispensing the use of expensive thermal cycler. Very recently, RPA was developed and evaluated for specific diagnosis of banana bunchy top virus, in different banana cultivars (Kapoor *et al.*, 2017). Of the total 133 symptomatic as well as asymptomatic banana leaf samples from various cultivars evaluated from the different regions of India, 106 samples (98-symptomatic, 8-asymptomatic) were found positive for BBTV infection in RPA assay. BBTV was efficiently detected using crude leaf sap in RPA and the results obtained were in consistent with PCR-based detection using purified DNA as template (Fig. 15). An attempt has also been made to detect CMV using fluorescence based reverse transcription-recombinase polymerase amplification (RT-exo-RPA) assay. A total of 120 banana plant samples collected from banana orchards located in Jalgaon, Maharashtra, India were evaluated, of which 62 symptomatic and 8 asymptomatic samples tested positive in basic RT-RPA as well as RT-exo-RPA assay using crude leaf sap template. Similar results were observed in symptomatic samples with RT-PCR

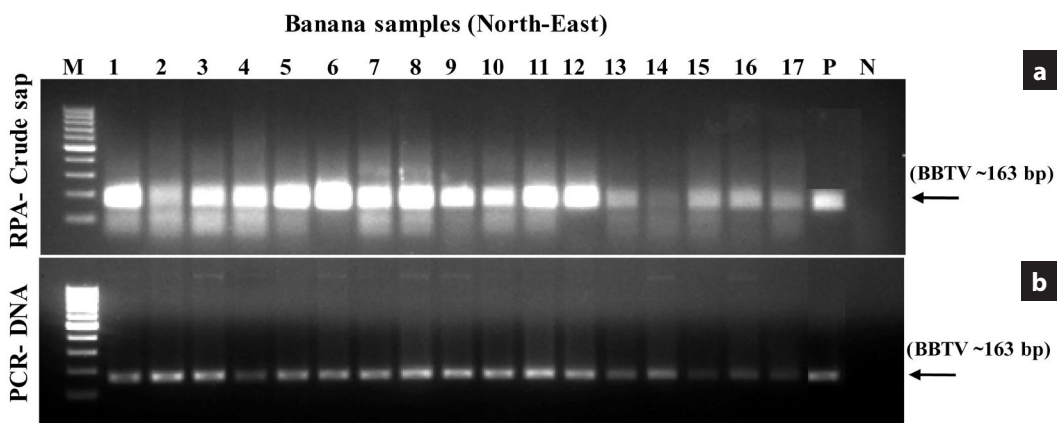


Fig. 15. Detection of banana bunchy top virus in banana leaf samples by RPA using crude leaf sap and by PCR using purified DNA template. Lane 1-17-Test samples, Lane P- Positive control (BBTV infected banana leaf); Lane N-Negative control (healthy banana leaf); M = 100 bp DNA ladder (Source: Referral Centre for Virus Indexing, IARI, New Delhi).

using purified RNA template (Fig. 16). The results varied with a few asymptomatic samples (13 samples) which tested positive in RT-PCR using purified RNA template but were found negative for CMV using RPA assays with crude sap template. The failure to detect CMV in asymptomatic samples using crude sap template in RPA assay was mainly due to extremely low virus titre in the samples (Srivastava *et al.*, 2019). RPA does not require purified DNA/RNA template and can be easily performed using a very small amount of crude sap extract. In comparison to conventional PCR/RT-PCR which takes around 4-6 hours for analysis, RPA amplification and analysis of amplicons can be easily done in less than one hour. The rapid speed, sensitivity and simplicity of RPA makes it an ideal technique for plant virus indexing on a large scale. In the near future, it can be successfully employed for mass indexing of banana viruses for the certification programme.

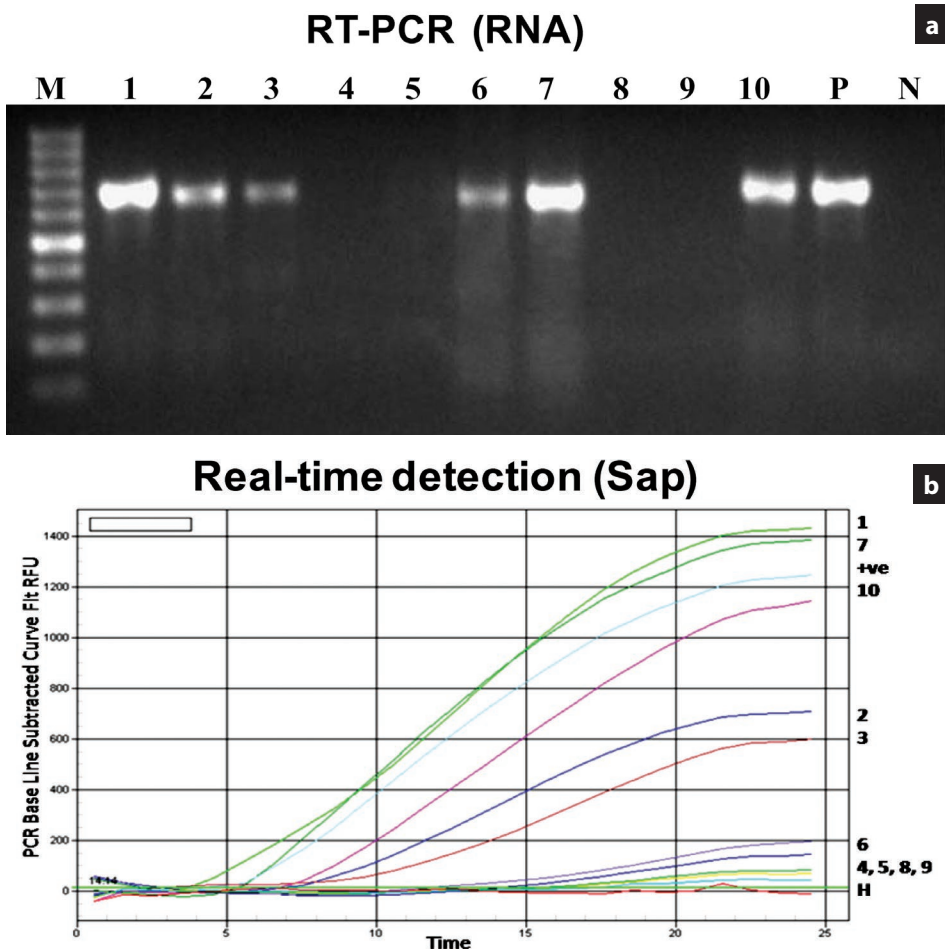


Fig. 16. (a) Detection of cucumber mosaic virus in banana leaf samples by RT-PCR using purified RNA template and **(b)** by RT-exo-RPA using crude leaf sap. Lane 1-10- Test samples, Lane P- Positive control (657 bp, CMV infected banana leaf); Lane N-Negative control (healthy banana leaf); M = 100 bp DNA ladder. (Source: Referral Centre for Virus Indexing, IARI, New Delhi).

10 Challenges

10.1. Certification procedure

Cultivation of banana through tissue culture has gained immense popularity in India owing to the several advantages over conventional sucker propagation. However, tissue culture does not always guarantee the exclusion of viruses. Prior to mass multiplication, virus testing of every mother plant following the standard procedure is of utmost importance. The viruses can easily transmit through the mother clones and hence every mother plant/stock culture need to be tested for the freedom from viruses. Currently, certification of banana involves the virus indexing of four viruses that are reported in India viz, BBTv, BSMYV, CMV and BBrMV, through standardized immuno- and nucleobased tests developed under the NCS-TCP system. During indexing, even if a single plant is found positive for a particular virus, the entire batch needs to be destroyed. Under NCS-TCP, a maximum of 10 mother plants/stock cultures may be pooled for testing. If the virus titre is low in a particular plant, pooling may further negate the test result.

Another challenge lies in addressing the new viruses that can enter through globalization and international trade of banana crop. There are other viruses of less significance such as abaca mosaic virus, abaca bunchy top virus, banana mild mosaic virus and banana virus X, known to occur in banana in different countries. In India, banana mild mosaic virus was first reported by Selvarajan and Balasubramanian (2016) and has yet not been included for testing under certification as its economic impact is not yet established. In case of banana streak viruses, five major banana-infecting badnavirus (BIB) species have been identified so far which include banana streak GF virus (BSGFV), banana streak MY virus (BSMYV), banana streak OL virus (BSOLV), banana streak VN virus (BSVNV) and banana streak IM virus (BSIMV). Of these five BIB species, currently only BSMYV which is the most commonly infecting badnavirus in India is being indexed. An enormous challenge in BSV indexing is discriminating the integrated sequences from the cognate episomal virus. BSV sequences present in the genome of *Musa balbisiana* hinder the detection of cognate episomal viruses by PCR since, PCR-based detection in hybrid banana varieties often leads to false positives due to amplification of integrated viral sequences. Techniques like immunocapture PCR (IC-PCR) and rolling circle amplification (RCA) have been developed for reliable diagnosis of episomal virus but have their own limitations and are not suitable for mass indexing. In case of CMV and BBrMV, indexing through serological based ELISA method is not always accurate in leaf samples which have low virus titre. ELISA is less sensitive than PCR-based detection and hence, there are chances of false negatives which need to be addressed.

10.2 Fungal and bacterial diseases

There are several bacterial (Bacterial wilt, *Erwinia* rot) and fungal (Anthracnose, Sigatoka, Cigar end rot) diseases affecting banana plantation and timely management of these diseases using standardized methods are often not practised by the farmers which lead to huge loss in profitability of banana TC production. Recently, there is an emergence of new disease, Fusarium wilt caused by the soil-borne fungus *Fusarium oxysporum* f. sp. *cubense*. Race 1, 2 and 3 affect relatively small number of banana cultivars and Race 4 is known to predominantly attack Cavendish, the most widely cultivated variety in the world. Tropical Race 4 (TR4) infests banana plants with the fusarium wilt disease through the roots that spreads to the vascular system and blocks transportation of water and nutrients in the stem, resulting in yellowing of leaves and plant death. Fusarium TR4 has infested banana plantations in South-East Asia and Pakistan, and has more recently been reported in Jordan, Mozambique and Australia. TR4 has also hit banana plantations in India. It first surfaced in the Katihar and Purnea districts of Bihar in 2015 and has since spread to the states of Uttar Pradesh, Madhya Pradesh and Gujarat (<https://www.thehindubusinessline.com/economy/agri-business/india-in-a-race-against-wilt-in-cavendish-banana/article23650060.ece>). It is a growing concern for the industry as it mainly destroys Cavendish banana plants and virtually all export bananas as well as the majority of bananas cultivated for own consumption or local markets are Cavendish bananas or other cultivars susceptible to Fusarium TR4. Once infected, the disease cannot be controlled by common chemical or cultural management practices. The fungal resting spores can remain viable in the soil for decades. Currently, there are no resistant varieties that can replace the export Cavendish banana, the only preventive measure is quarantine (FAO, 2019 <http://www.fao.org/world-banana-forum/projects/fusarium-tr4/disease/en/>).

10.3. Plantlet production and distribution

The timely production of tissue culture plants for different regions is critical. Surplus production of TCP at the time when demand is less can lead to huge loss to TCPFs.

10.4. Technology transfer

The protocol for tissue culture production of banana is well standardized but for a start-up TCPF, it can be challenging to adhere to the standard practices. In the case of non-compliance to the standard package of practices, there could be high degree of contamination during initiation and multiplication which can lead to huge loss.

10.5. Adoption by farmers

The tissue culture cultivation of banana is the most promising method for mass production of quality, uniform, disease free planting material in a short period of time. Its application, however, is limited mainly due to the high initial cost of plantlets and lack of awareness of the technology.

Over the time, there is rapid increase for banana and the demand for new planting material is also increasing fast. The conventional clonal propagation methods cannot cope up with the continuous demand for new planting material. Tissue culture technology is superior to conventional propagation in terms of optimal yield, uniformity, disease free planting material and true to type plants. However, despite the several advantages of TC banana plants, the initial high cost of plantlets is one of the major pitfalls in adoption of TC technology. Also, the lack in reliable market facilities, lack of expertise about identifying the pests, diseases and knowledge of plant protection and non-availability of genuine planting material are problems in tissue culture banana. Tissue culture banana plants are sold at an average price of ₹ 14 as compared to traditional planting material (suckers) costing ₹ 2-3/unit (Shukla, 2016).

In the Jalgaon district of Maharashtra, a major banana growing region, tissue-cultured banana was found to be more profitable to farmers than sucker-propagated banana (Bachchan, 2016). A sample of 60 farmers from selected villages were randomly chosen and the data was collected in the agricultural year 2010-2011 by personal interview. The per hectare yield in TCB was 60.50 metric tonnes which was higher than the yield of sucker banana (45.50 metric tonnes). By products of banana included suckers and leaves. The gross returns obtained per hectare of TCB were ₹ 4,58,991 which was 33.22% higher than the gross returns obtained from sucker banana (₹ 3,44,522). The net returns obtained in sucker propagated banana (SPB) were ₹ 1,81,216 and in TCB were ₹ 3,01,605.

Similarly, in Karnataka, TCB was beneficial over SPB to the banana growers (Hanumantharaya *et al.*, 2009). The data was collected in the agricultural year 2005-2006 from 80 farmers (40=sucker banana growers and 40=tissue culture banana growers) in 12 villages of two taluks in Tungabhadra and Malaprabha command areas of Karnataka. Results of the study revealed that, in crop-I, per ha production cost of sucker banana was ₹ 82,298 and TCB was ₹ 1,17,563. This was mainly due to high cost of TC plantlets (₹ 34,000) than cost of sucker (₹ 7,340.6) and also higher utilization of plant protection chemicals and labours in the case of TCB than that of SPB. The gross returns obtained for TCB were ₹ 1,97,295.94 which was 23.22% higher than the gross returns from sucker banana (₹ 1,60,113.81). The net returns obtained were ₹ 77,815.81 and ₹ 79,732.94. In crop-II, not much difference was observed in production cost of sucker and TCB with respective

figures of ₹ 55,073 and ₹ 57,561.30. This was due to similar operations in both the methods of production. The gross returns realised were ₹ 1,70,596.56 for sucker and ₹ 1,85,953.07 for TCB (9% higher) respectively and the net returns were ₹ 1,15,523.56 and ₹ 1,28,391.77, respectively.

In Theni district of Tamil Nadu, the farmers found TCB to be cost effective in comparison to the sucker-propagated banana (Alagumani, 2005). The data was collected by personal interview of randomly selected 60 sample farmers who raised banana through suckers and 30 farmers who used tissue-cultured plantlets. The total cost of cultivation of TCB and SPB were ₹ 141040 and ₹ 108294 per hectare, respectively and it was higher for TCB by 30.24% due to high plantlet cost. The gross income obtained was higher by 35.35% in TCB than SPB, which was ₹ 2,53,302 and ₹ 1,87,149 per hectare, respectively. Gross income and bunch weight are the major factors influencing the adoption of TCB.

Despite the high initial cost of investment, the farmers should be encouraged to adopt TCB to get higher yield and economic profits.

11.1 Export status of banana

Banana is the maximally produced and consumed amongst the fruits cultivated in India and accounts for about 32% of the total fruit production in India as per the All India Third Advance Estimates of Area & Production of Horticulture Crops, Ministry of Agriculture & Farmers Welfare during 2017-18. India ranks first both in terms of area and production of banana in the world. The five important banana producing states in the country (Maharashtra, Tamil Nadu, Gujarat, Karnataka and Andhra Pradesh) contribute more than 70% of total banana production in the country. Maharashtra is the largest banana exporting state with 96.91% share in value. Maharashtra supplied bananas worth USD 47202673 to the countries, especially Middle East in 2017. In 2018, Tamil Nadu started the export of long-shelf, high-quality bananas to Europe. Developed with an Italian technology, the first batch of the 'Grand naine' range of bananas was exported to Italy and subsequently to other countries in Europe. About 21 tonnes of banana harvested from various parts of Tamil Nadu were exported to Italy from the Kochi port (<https://www.thehindu.com/news/cities/Coimbatore/tn-begins-exporting-bananas-to-italy/article25383642.ece>). Despite being the world leader in banana production, India occupies the 20th position among the top banana exporting countries. This may be due to the fact that large volumes of bananas are also consumed in the country, in addition to this, India's post-harvest facilities are not fully developed including the poor cold chain infrastructure. According to the export numbers by APEDA, India exported 1,01,314.33 MT of banana worth ₹ 3487.742 million in 2017-18. Maximum banana from India is exported to Middle East countries in which the United Arab Emirates, Oman, Saudi Arabia, Iran and Kuwait are among the top export destinations. Jawaharlal Nehru

Port (JNPT), also known as Nhava Sheva recorded the highest export shipments of banana worth USD 16900646 during 2017 (<https://www.exportgenius.in/blog/exports-of-banana-from-india-in-2017-indias-top-exporters-of-banana-246.php>). Fig. 17 below depicts the export process of banana.

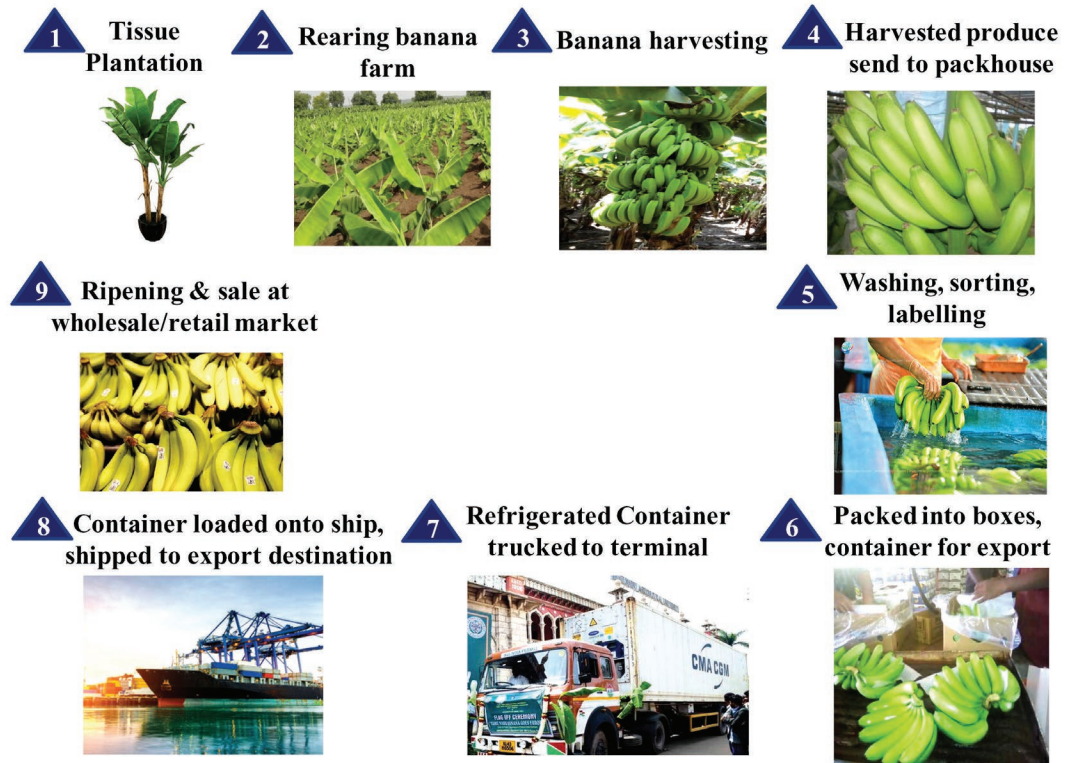


Fig. 17. Flowchart depicting the export process of banana

12 Benefits to Farmers Using Certified Tissue Culture-raised Banana

Mr Ram Saran Verma, popularly referred as the Banana King of Uttar Pradesh is one of the first farmers to introduce TC in banana farming way back in 1988. He initially planted the crop on one acre land and attained profits of ₹ 0.4 million. The 1-acre plantation yielded 0.4 MT of bananas. While the cost of production was about ₹ 0.1 million at the end of 14 months, the farmer earned a profit of over ₹ 0.4 million *i.e.*, 4x higher than his initial investment. TCB plantation at 1,100 plants per acre with a distance of 6' × 6' has been perfected by him. Per plant expenditure comes around ₹ 60-70 and gross income is of ₹ 300. His methods were adopted by other farmers and nearly about 50,000 farmers from neighbouring districts have been benefited. The crop ensures a net profit of ₹ 0.20 to 0.25 million on every acre of land and hence more farmers are opting for banana cultivation using TC plants (<https://www.thebetterindia.com/165736/uttar-pradesh-banana-farmer-news/>).

Mr B. Venkatasamy, a farmer in Onnalvadi near Hosur in Karnataka was attracted towards banana cultivation through tissue culture. He cultivated G9 variety through TC technology provided by the Horticulture Department of state government using the drip irrigation system. He got subsidy from the government under the National Horticulture Mission. He invested ₹ 75.0 a plant on 4.5 acres and after eleven-and-a-half months, the total profit earned from cultivation of banana on 4.5 acres was around ₹ 19 lakhs. This was the highest return ever he got during his 25 years of banana cultivation practices. This further encouraged him to cultivate G9 variety on another 10 acres. (<https://www.thehindu.com/todays-paper/tp-national/tp-tamilnadu/Record-harvest-of-banana-through-tissue-culture/article16474560.ece>).

Tissue culture has helped **West Bengal** make banana cultivation an attractive proposition for farmers. Since its adoption about five years ago, TC banana cultivation has increased significantly in West Bengal. The banana cultivators got a higher price for their crop, ₹ 10.50 per kg on an average from ₹ 7 per kg in 2011. **Mr Isad SK** from Nadia and **Mr Nurul Islam Mondal** from Murshidabad district confirmed that the G9 variety of bananas propagated by using TC plants resulted in about 40% higher income than the traditional variety called Robusta. The Keventer Agro Ltd. is the major company behind the enhanced TC banana production in West Bengal. They sold 19,000 tonnes of bananas in September 2017 whereas 3,656 tonnes banana were sold in 2013-14 (<https://economictimes.indiatimes.com/news/economy/>

agriculture/how-tissue-culture-is-helping-bengals-banana-production/articleshow/60977613.cms).

In **Maharashtra**, almost 130,000 hectare come under banana cultivation out of which more than 50% is in Jalgaon and adjoining area. Traditionally, banana was planted with suckers and the farmers could get average yield of 13 kg/plant in 15-18 months. Jain Irrigation Systems in Jalgaon introduced TC-raised banana plants and pioneered tissue culture of 'Grand naine' variety of banana since 1994-95. It is the largest banana TC laboratory in India and has successfully micro-propagated and sold over 2 million G9 banana plants with outstanding success. Through TC, the farmers could increase their productivity from an average of 12 kg to over 26 kg per bunch per year and have consecutively taken crops with two ratoons in about 30 months. Economics in terms of cost of production and net profits of some of the banana growers in Maharashtra have been shown in Table 8.

These farmers declared the net profit of ₹ 0.24 million/acre to 0.325 million/acre, which is the highest income possible for an annual crop (11 month) in India. These were the result of good quality planting material, agri-advisory services of Jain Irrigation System Pvt. Ltd. drip irrigation and fertigation as per crop requirement (Dafare *et al.*, 2014).

The banana growers in Tamil Nadu were earning revenue of about ₹ 50,000 per acre by following the traditional methods of cultivation. After planting the TC plants and adopting the scientific method of farming (drip irrigation, fertigation and post-harvest management), the income of banana farmers progressively increased by 6x from ₹ 50,000 to over ₹ 3,00,000 per acre (Press Release, 2018, DIPR, Secretariat, Chennai).

There has been a tremendous impact of banana tissue culture farming on profitability and improvement in livelihood of banana growers. It has enabled to increase in the income of the farmers by 5-6 fold and with further development in post-harvest facilities and cold storage infrastructure, there is huge scope to bring more area under banana cultivation for export purposes.

Table 8. Net profit of one season by some banana growers in Maharashtra

S. No.	Name of farmer	District	Area (acre.)	Plant spacing (feet)	No. of Plants / acre	Average cost of cultivation (₹/ac.)	Market price (₹/ kg)	Average yield (kg/ acre)	Gross return (₹/ acre)	Net profit (₹/acre)
1.	D.R. Mali	Nandurbar	11	6*5	1452	94380	10.0	40656	406560	312180
2.	T.M. Borade	Pune	1.5	7*5	1250	115000	10.0	40000	400000	285000
3.	P.H. Mahajan	Jalgaon	7.3	5.2*5.5	1508	98020	6.5	52780	343070	245050
4.	P.D. Deshmukh	Jalgaon	18	5*5.5	1584	110880	10.0	42768	427680	316800
5.	D.R. Bhawal	Ahmad Nagar	2.5	6*5	1452	108900	8.5	46404	394434	285534
6.	A.K. Patil	Buldana	4.25	5*5	1742	137500	8.0	57486	459888	322388

Source: Dafare et al. (2014) Jain Irrigation Systems Ltd

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Asia-Pacific Association of Agricultural Research Institutions

Asia-Pacific Association of Agricultural Research Institutions (APAARI) was established in 1990 at the initiative of Food and Agriculture Organization of the United Nations and most of the National Agricultural Research Systems (NARS) of the Asia-Pacific region. Its mission is to promote the development of National Agricultural Research Systems in Asia-Pacific region through facilitation of inter-regional, inter-institutional and international partnerships.

APAARI's vision is that Agricultural Research for Development (ARD) in the Asia-Pacific region is effectively promoted and facilitated through novel partnerships among NARS and other related organizations so that it contributes to sustainable improvements in the productivity of agricultural systems and to the quality of the natural resource base that underpins agriculture, thereby enhancing food and nutrition security, economic and social well being of communities and the integrity of the environment and services it provides.

The overall objectives of APAARI are to foster the development of agricultural research in the Asia-Pacific region so as to:

- Promote the exchange of scientific and technical information
- Encourage collaborative research
- Promote human resource development and capacity building
- Build up organizational and management capabilities of member institutions
- Strengthen cross-linkages and networking among diverse stakeholders

APAARI's strategic thrusts are:

- Building research partnerships
- Regional research networking
- Policy advocacy for ARD
- Information dissemination
- Human resource development
- Technology transfer

To know more about APAARI, please visit: <http://www.apaari.org>

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- *In Vitro* and Cryopreservation Approaches for Conservation of Plant Genetic Resources: Training Manual (2019)
- International Hands-on Training on Genome Editing Technology: Training Manual (2019)
- Regional Workshop on Underutilized Animal Genetic Resources and their Amelioration: Proceedings and Recommendations (2019)
- Regional Workshop on Underutilized Animal Genetic Resources and their Amelioration: Regional Status Reports and Strategic Papers (2019)
- Training on Transformation of Agricultural Education through Knowledge Management and Capacity Development for More Effective Agricultural Innovation System (AIS) (2019) (softcopy)
- Regional Workshop on Knowledge Management for More Effective Agricultural Innovation Systems (AIS) (2018) (softcopy)
- Regional Expert Consultation on Agricultural Biotechnology – Scoping Partnerships to Improve Livelihoods of Farmers in Asia and the Pacific: Strategic Papers and Country Status Reports (2018)
- Regional Expert Consultation on Agricultural Biotechnology – Scoping Partnerships to Improve Livelihoods of Farmers in Asia and the Pacific: Proceedings and Recommendations (2018)
- Regional Expert Consultation on Underutilized Crops for Food and Nutritional Security in Asia and the Pacific: Thematic, Strategic Papers and Country Status Reports (2018)
- Regional Expert Consultation on Underutilized Crops for Food and Nutritional Security in Asia and the Pacific: Proceedings and Recommendations (2018)
- Investment in Agricultural Research for Sustainable Development in Asia and the Pacific: Country Status Reports (2017)
- Scaling Conservation Agriculture for Sustainable Intensification in South Asia – Proceedings and Recommendations (2017)
- Expert Consultation on Best Practices in Agri-food Innovations in Asia and the Pacific – Proceedings & Recommendations (2016)
- 14th General Assembly, 3 November 2016 – Proceedings
- High Level Policy Dialogue on Investment in Agricultural Research for Sustainable Development in Asia and the Pacific, 8-9 December 2015 – Proceedings
- The Executive Committee Meeting of APAARI, 8 September 2016 – Proceedings
- The Executive Committee Meeting of APAARI, 10 December 2015 – Proceedings
- Workshop on Development of Communication Strategies for Adoption of Agri-Biotechnology in the Asia-Pacific Region, 28-29 September 2015 – Proceedings
- Capacity Development Workshop On Planning, Monitoring and Evaluation towards Measuring Outcomes and Impacts, 3-7 August 2015 – Proceedings
- Expert Consultation on Assuring Food Safety in Asia-Pacific, 4-5 August 2014 – Proceedings
- 13th General Assembly Meeting, 1 November 2014 – Proceedings
- 12th Asian Maize Conference 'Maize for Food, Feed, Nutrition and Environmental Security', 30 October-1 November 2014 – Proceedings
- Expert Consultation on Promotion of Medicinal and Aromatic Plants in the Asia-Pacific Region, 2-3 December 2013 – Proceedings
- Asia-Pacific Symposium on Molecular Breeding 1-3 October 2013 – Proceedings
- Regional Workshop on Youth and Agriculture: Challenges and Opportunities : Proceedings (2013)

- Expert Consultation on Strengthening Linkages between Research and extension to Promote Food and Nutrition Security : Proceedings (2013)
- NARS-CGIAR Interactive Session for Strengthening Partnership in South Asia : Proceedings (2013)
- National Workshop on Outscaling Farm Innovation : Proceedings (2013)
- International Conference on Innovative Approaches for Agricultural Knowledge Management: Global Extension Experiences: Proceedings (2013)
- Training Workshop on Open Access Publishing Using Open Journal Systems: Proceedings (2013)
- Foresight and Future Pathways of Agricultural Research Through Youth: Proceedings and Recommendations (2013)
- Expert Consultation on Managing Trans-boundary Diseases of Agricultural Importance in the Asia-Pacific: Proceedings and Recommendations (2013)
- Stakeholders' Dialogue on Biosafety Regulations in the Asia-Pacific Region Proceedings and Recommendations (2013)
- Regional Consultation on Agricultural Research for Development : Proceeding and Recommendations (2013)
- Regional Consultation on Collective Actions for Opening Access to Agricultural Information and Knowledge in the Asia-Pacific Region: Proceedings (2012)
- Prioritization of Demand-driven Agricultural Research for Development in South-Asia (2012)
- Regional Consultation on Improving Wheat Productivity in Asia: Proceedings and Recommendations (2012)
- Workshop on Climate-Smart Agriculture in Asia: Research and Development Priorities: Proceedings and Recommendations (2012)
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- Regional Workshop on Implementation of Suwon Agrobiodiversity Framework: Proceedings (2012)
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- Expert Consultation on Agricultural Biotechnology, Biosafety and Biosecurity: Proceedings and Recommendations (2012)
- Workshop on Moving Beyond Strategy to Improve Information and Knowledge Management for Agricultural Development in the Pacific Islands Countries and Territories: Proceedings (2012)
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Success Stories

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- Hybrid Rice in China - A Success Story (1994), Lou Xizhi and C.X. Mao
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