STUDIES ON BANANA BUNCHY TOP DISEASE

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IN

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By

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CERTIFICATE

This is certify that the thesis entitled "**STUDIES ON BANANA BUNCHY TOP DISEASE**" *submitted by* **AMBIKA, D.S.** *for the degree of* **MASTER OF SCIENCE (AGRICULTURE)** *in* **PLANT PATHOLOGY**, *to the University of Agricultural Sciences, Dharwad, is a record of bonafide research work done by her during the period of her study in this University, under my guidance and supervision and the thesis has not previously formed the basis of the award of any degree, diploma, associateship, fellowship or other similar titles.*

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DEDICATED TO MY BELOVED

PARENTS

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INTRODUCTION

I. INTRODUCTION

Banana (*Musa* spp.) is one of the most important tropical fruit crops of the world. It is an important economically profitable plantation crop of India after mango, having high export potential. It is the fourth important global agricultural commodity in terms of gross value of the produce after rice, wheat and milk.

Banana fruits are available throughout the year, unlike other fruits that are seasonal. With its high nutritive value and low market price, it is popularly known as 'poor man's fruit'. It is also known as 'Apple of paradise' for its medicinal values. Banana originated in Indo-China and South East Asia, where the earliest domestication is considered to have occurred (Simmonds, 1966). It is grown in more than 120 countries across the tropical and subtropical zones of the world. It provides livelihood to millions of people and also a major source of food for over hundred million people in the hot, low land tropics of Asia, Africa and America. Bananas for export account for only about 10 per cent of total production. The overall production can be divided into two main categories in which dessert banana constitutes 43 per cent and cooking banana, 57 per cent of the world production.

Banana by virtue of its multiple uses is popularly known as 'Kalpa taru'. Apart from consumption, the leaf is used as biological plate for serving food. Dessert banana has a therapeutic value. In Kerala, one of

the cultivars is used as baby food, Paperboard, plywood, tissue paper, etc. can be prepared out of banana pseudostem and it is also a major source of fiber extraction in Brazil. Various value added fast food products like banana chips, fig, soft drinks, confectioneries, dehydrated core products fortified wine, spirit, beer and many of the alcoholic products are made out of banana.

The fat content of bananas and plantains is very low, while carbohydrate content contributes to about 95 per cent of the total energy of the food. The fraction of energy contributed by the fat is only one to two per cent. Similarly, protein contribution is low at three per cent of total energy. The ripen banana contains a fair amount of vitamins A, B_1, B_2 and C, small quantity of vitamin D and E and also a large amount of potash, phosphorous, calcium and iron. Banana is a rich source of energy, about 24 bananas each weighing around 100 gm provide the energy requirement (2400 cal/day) of a sedentary man. They are also seen as natural convenient, ready, wrapped and healthy snake food.

Banana is a widely distributed fruit crop of India. The long period of its domestication is evident by its mention in Koutilya's Arthasastra (250-300 BC) and other ancient scriptures and epics. India is also the home of multitudes of varieties. Though an array of varieties are grown with regional preference, a group of Cavendish, Rasthali, Poovan and Nendran are the basis of commercial cultivation (Tajuddin *et al.*, 1996).

The area under banana crop in the world is 5,31,89.14 thousand ha with production of 58,97.40 thousand tonnes and productivity of

19.4 tonnes/ha. India accounts for 14 per cent of world production. Banana, known for its antiquity, is having great socio-economic significance in India, with a total annual production of 16.91 m tonnes from 490.70 thousand ha. Banana contributes to 37 per cent of fruit production in India.

The major banana growing states are Maharashtra, Tamil Nadu, Karnataka, Gujarat, Madhya Pradesh, Bihar, Orissa, Andhra Pradesh, Assam, Kerala and West Bengal. In Karnataka banana is grown in 58,721 ha area with total production of 12,35,153 tonnes (Anon., 2001). With increasing demand for banana in market, the constraints in its cultivation are also increasing.

In India, banana crop is affected by several diseases caused by fungi, bacteria, nematodes, viruses and abiotic diseases. Among these, diseases caused by viruses like bunchy top, banana streak, infectious chlorosis, bract mosaic, mild mosaic, dieback, abaca mosaic and enset streak are important.

Bunchy top was first reported from Fiji in 1889 (Stover, 1972) and in India for the first time from Kerala in 1940 (Mehta, 1964). The disease is prevailing in almost all banana-growing areas of the country. The losses are 100 per cent in yield as in this case no bunch is formed. In Karnataka also similar loss is found but no authenticated studies or reports so far have been made or published. Hence looking to the

economic importance of the disease the present investigation was undertaken with the following objectives.

1. Survey to know the incidence of banana bunchy top in Belgaum, Dharwad and Haveri districts.
2. Studies on symptomatology.
3. Transmission of Banana Bunchy Top Virus (BBTV) by mechanical inoculation, aphids and plant material.
4. Standardization of serodiagnostic technique.
5. Management of BBTV through meristem tissue culture technique.

REVIEW OF LITERATURE

II. REVIEW OF LITERATURE

Bunchy top of banana is a serious viral disease and is also known as cabbage top, curly top or strangles. It is widely distributed in many countries such as Fiji, Pacific Islands, Australia, Egypt and the East. The disease was first observed in Fiji in 1889 (Stover, 1972). In 1913, it was reported from Ceylon and from Australia in 1920 and more recently from Pakistan in the early 1990's. The disease has been identified in the Pacific region (including American Samoa, Australia, Hawaii, Kiribati, Ogosowala-gentu, Tonga, Tuvalu, Wallis Island and Western Sumoa), Asia (including China, India, Indonesia, Pakistan, Philippines, Sri Lanka, Taiwan and Vietnam) and Africa (including Burundi, Congo, Central African Republic, Egypt, Gaban, Rwanda and Zaire). The occurrence of bunchy top in Kundang, Sleonga and Bulit Merah, Penang of Malaysia was also noticed by Doon (1991).

In India it was first reported from Travancore in 1940 (Mehta, 1964). The disease has now spread to Andhra Pradesh, Tamil Nadu, Orissa, Maharashtra, Bihar, Karnataka, West Bengal, Assam and Uttar Pradesh (Varasi, 1959; Capoor, 1967; Singh 1979). A loss of about rupees 40,000,000 annually was reported in Kerala alone (Mehta, 1964). Among the eight viruses infecting on banana, Banana Bunchy Top Virus (BBTV) was found top in damaging the plant and total yield of the crop as it does not allow for flower setting and fruit formation. Kesavamurthy (1980) reported that more than 60 per cent of the areas in lower Palani hills of Madurai district was completely devastated by BBTV.

2.1 BANANA BUNCHY TOP VIRUS

Banana Bunchy Top Virus (BBTV) is a single stranded DNA virus circular in nature having three parts of which largest genome part is of 1.3 kb length. Virus is iosmetric, nonenveloped, 18 to 20nm in diameter, round in profile; without a conspicuous capsomere arrangement. It is highly intensified in phloem region of the host. In nucleic acid hybridization test, its genome reacts distinctly with that of abaca bunchy top virus. It was originally thought to be a luteovirus; infected plants sometimes contain dsRNA, but these may come from a contaminant virus. The virus infects mainly on Musaceae family host with unique symptoms of chlorosis, stunting and death of the infected plant (Dale, 1991). Burns *et al.* (1995) reported that an additional 5 circular ssDNA components of BBTV which were designated components 2, 3, 4, 5 and 6 were cloned, sequenced and analysed. These components were present in all BBTV infections tested. They concluded that BBTV belongs to an undescribed plant virus group which could also include subtarraneas clover stunt virus, coconut foliar decay virus, faba bean necrotic yellows virus and milk vetch dwarf virus.

2.2 SURVEY FOR THE INCIDENCE OF THE DISEASE

In a survey in the central highlands of South Vietnam, Vakili (1969) observed that most of the bunchy top infected areas occurred on the west side of the Annamitique or Truong san chain of mountains stretching north-south and the middle of south vietnam crescent. Govindaswamy *et al.* (1977) recorded 10 to 40 percent incidence of banana bunchy top disease in 10 villages of lower Pulney hills and the maximum of 80 per cent was in Agamalai area. Kadhirvel *et al.* (1986) reported 20 to 80 per cent incidence of banana mosaic in cv. poovan from Trichy district in Tamil Nadu.

Thiribhuvanamala and Doraiswamy (2001) surveyed the major banana growing areas of Tamil Nadu to assess the incidence of the four viruses (BBTV, BBMV, BSV, CMV). Their survey data revealed higher incidence of (26.4%) banana bunchy top disease in Thadiyankudisai in Dindigal district of Tamil Nadu.

Singh and Verma (2002) during their survey in banana growing areas of Maharashtra recorded 5.2 to 21.2 per cent incidence of banana infectious chlorosis caused by cucumovirus, 0.62 to 2.25 per cent incidence of banana streak caused by badnavirus and 4.1 per cent incidence of banana bract mosaic caused by potyvirus. In another survey conducted at 20 villages of Marathwada region of Maharashtra, Kenganal (2003) recorded 6.6 percent, 6.1 per cent and 5.0 per cent incidence of Banana Streak Virus in tissue cultured, ratoon and sucker plantations of Banana respectively.

Manoranjitham *et al.* (2003a) found 84.5 percent of BSV in Trichy district of Tamil Nadu and more than 70.0 per cent in Tuticorin, Cuddalore, Karur and Erode districts. Kunkalikar *et al.* (2003) reported incidence of banana bract mosaic virus up to 90 per cent in banana plantations in and around Dharwad district.

2.3 SYMPTOMATOLOGY

In the early history of BBTV symptoms, Alagiamanavalan *et al.* (1973) observed that affected plants were highly stunted with leaves reduced in size, chlorotic, brittle with upright growth habit. They appeared crowded at the throat region. Vakili (1969) observed characteristic vein clearing and deformation of lamina and presence of thin dark green streaks on the lamina of garden plants of canna, infected by abaca strain of the virus.

Singh (1979) observed that artificially BBTV inoculated plants did not show any difference with naturally infected ones. In case of young seedlings emerging from infected planting material, plants were markedly stunted and grow to a maximum height of one to three feet depending on the variety and not flower or fruit. Affected plants show narrow chlorotic leaves with pale and ragged margins which emerge only partially thus farming a bunch at the apex of the plant. Owing to the failure of the leaf stalks to emerge, or to come out of the pseudo-stem in most of the cases, the leaves stand more erect than in normal. The infected leaves become brittle both in young as well as older plants. Khalid and Soomro (1993) observed symptoms on samples collected from diseased plants, which had short, erect leaves, severe stunting and pronounced bunchy top appearance and also recognized by their upright leaves and reliably diagnosed by the presence of dark-green streaks in the leaf veins

Gauhl and Pasberg (1994) observed important symptoms of BSV such as chlorotic streaks, necrotic streaks, stunting of plants and reduction in the petiole length of the leaves. Kenganal (2003) observed various kinds of symptoms *viz.* chlorosis, necrotic streaks on leaf and stem, internal necrosis of pseudostem, distortion of leaf lamina, mosaic appearance, stunting of plants without bearing any fruits etc. on banana streak diseased plants. A variation in symptom expression was also recorded related to changes in environmental conditions. Kunkalikar *et al.* (2003) noticed that BBrMV infected plants produce dark reddish brown mosaic pattern on the bracts of inflorescence.

2.4 TRANSMISSION

Khurana (1971) observed that bunchy top disease was not mechanically transmissible using diseased leaf sap and inoculating with the usual leaf rubbing method. He also noticed that inoculation using aphids under glasshouse conditions did not show any success in transmitting the virus, but in case of field conditions he observed the symptoms of BBTV on plants colonized with aphids. Nair *et al.* (1973) in their field studies they found that banana aphid *Pentalonia nigronervosa* Coq. was the vector of the virus causing bunchy top disease in banana.

Raj *et al.* (1970) found that when BBTV was inoculated at the neck region of banana plants with the help of the vector *P. nigronervosa* Coq. It took a minimum of five days to start moving inside the plant and if inoculated portion was removed, within five days the virus did not multiply inside the host.

Singh (1979) reported that BBTV could not be transmitted mechanically using sap from systemically infected plants. However, banana aphid, *P. nigronervosa* transmitted the causal agent successfully after an acquisition feeding period of 20 hours and inoculation feeding period of 2 hours. Regupathy (1980) observed that in perennial cultivation of banana under favorable weather conditions conducive for the aphids, the only known vector *P. nigronervosa* transmits the disease and help the disease to spread faster.

Lockhart (1986) studied the mode of transmission and found that neither streak disease nor BSV was transmitted mechanically from

infected banana to healthy banana or to any of the other test plants using crude sap or partially purified extracts. In glasshouse experiments, *Planococcus citri* and *Saccharococcus sacchari*, both mealy bugs known to colonise banana, and have been found capable of transmitting BSV (Lockhart, 1995). Hu *et al.* (1996) in their experiment found that an average of more than 65 per cent of single viruliferous adult aphids transmitted BBTV successfully. Nodwoara *et al.* (2000) confirmed the dissemination of BSV through vegetatively propagated planting material such as corms and suckers.

Thiribhuvanamala *et al.* (2001) conducted investigations on the virus vector relationship and revealed that pre-acquisition fasting for 15 minutes, acquisition feeding period of 24 hours and inoculation feeding period of 8 hours by viruliferous aphids resulted in cent percent transmission of bunchy top virus.

Manoranjithan *et al.* (2003b) found that mechanical inoculation of BSV to banana, rice, sugarcane, *Nicotiana glutinosa* and *Nicotiana benthamiana* did not produce any symptoms. Samples from these plants tested for the presence of BSV through TAS-ELISA, showed negative results. The virus was transmitted only through vector sugarcane Mealybug *Saccharicoccus sacchari*. Kenganal (2003) observed 100 per cent transmission of BSV by vegetative propagation planting material like suckers, tissue cultured plantlets derived from infected explants only.

2.5 SERODIAGNOSTIC TECHNIQUES

Various serodiagnostic tests are used in diagnosis of virus-infected plants such as ELISA, Direct Antigen Coating (DAC) ELISA, Double

Antibody Sandwich (DAS) ELISA, Immuno Sorbent Electron Microscopy (ISEM), Dot Immuno Binding Assay (DIBA), Tissue Immuno Binding Assay (TIBA) and Gel diffusion tests etc.

Voller *et al.* (1976) detected two morphologically different plant viruses using enzyme-linked antibodies.

Clark and Adams (1977) demonstrated characteristics of the microplate method of ELISA for the detection of plant viruses.

Wu and Su (1990a) raised the BBTV specific antibodies using mouse ascites and detected the virus from different samples using above antiserum in Plate Trapped Antigen (PTA) ELISA and Antibody Trapped Antigen (ATA) ELISA. Further they found ATA-ELISA was 16 times more sensitive than PTA-ELISA. This technique was also found capable of detecting the virus at a dilution of 1/512. Drew *et al.* (1992) tested the symptomless field plants by DAS-ELISA using a polyclonal antibody specific for BBTV. No detectable levels of virus were found in any of the samples. He concluded that symptomless plants are virus free.

Mariappan & Mathikumar (1992) found that the ELISA method is much more sensitive than the other methods and is helpful in identifying the infected plants even when the virus concentration was low. Where as Wu and Su (1992) using ELISA test detected BBTV even in the symptomless plants.

Hu *et al.* (1993) detected the BBTV in commercial banana (*Musa* spp.) plantations and residential areas on the Island of Oahu by double antibody sandwich direct ELISA with a monoclonal antibody ($3B_4$). Khalid and Soomro (1993) found positive results of ELISA with MAbs to a

Taiwanese isolate of BBTV. Kenyon and Brown (1997) used triple antibody sandwich enzyme linked immuno sorbent assay (TAS-ELISA) with poly and monoclonal antibodies specific for BBTV for detection of samples collected in the field. The samples from symptomatic plants gave positive and healthy samples negative reactions. Samuel *et al.* (1997) used plate trapped antigen (PTA) enzyme immuno assay to detect the BSV and BBrMV in banana samples.

Geering and Thomas (1997) compared serological tests for the detection of bunchy top virus in banana and among them, ELISA was adjudged the best. For the rapid detection of BSV Nodwora *et al.*, (2000) employed TAS-ELISA and suggested that the test could detect different isolates of virus.

2.6 PRODUCTION OF DISEASE FREE PLANTLETS USING TISSUE CULTURE TECHNIQUE

Tissue culture techniques are now a days widely used for production of disease free planting material. A rapid multiplication of bananas and plantains by *in vitro* shoot tip culture was developed by Cronauer and Krikorian (1984). Laxmikanth and Nataraja (1989) did a successfully regeneration of banana plantlets using meristem as explant. Wu and Su (1991) reported that BBTV infected tissue was cultured for 3 months at 35^{0}C. Some of the buds started to produce healthy looking plantlets. Five out of 11 cultures produced healthy looking plantlets in 6 months. Crude extracts of these plantlets did not react with monoclonal antibody prepared against BBTV, but his other studies revealed that the uneven distribution and low concentration of virus after high temperature

treatment leads to BBTV-free primordial cells which in turn developed healthy plantlets. Diekmann and Putter (1996) advised routine indexing of plants established from tissue culture.

Dahal *et al.* (1998) standardized protocol for obtaining BSV free material through a combination of thermotherapy and other methods of tissue culture techniques. Helliot *et al.* (2001) proposed the use of meristems excised from *in vitro* plants, *in vivo* plants and from highly proliferating meristems for the elimination of virus disease from *Musa*. Similarly they could obtain 30 per cent and 90 per cent virus free plants by using cryopreservation technique for CMV and BSV infected plants. The rouging is probably an adequate control measure for isolated out breaks. While selecting plants for propagation, all the leaves should be inspected for symptoms on several dates, including a date around the time of bunch initiation when symptoms expression appears to be the greatest (Daniells *et al.*, 2001).

Carpio (2002) revealed that mass production of healthy planting materials is the control approach to virus disease. These diseases commonly spread through propagative materials with widespread occurrence. Hence banana bunchy top virus (BBTV), free material can be obtained from field grown suckers as planting material in well monitored and roughed plots. Kenganal (2003) found that meristem culture alone or in combination with thermotherapy could not eliminate the BSV from the infected meristems.

MATERIAL AND METHODS

III. MATERIAL AND METHODS

Present investigation on banana bunchy top disease was carried out in Department of Plant Pathology and Department of Horticulture, University of Agricultural Sciences, Dharwad.

3.1 SURVEY FOR INCIDENCE OF DISEASE

A roving survey was undertaken in different villages of Belgaum, Dharwad and Haveri districts. The disease incidence was recorded at randomly selected banana orchards from different villages of these districts. The disease was diagnosed in the field based on symptoms present on the plants. The per cent disease incidence was recorded in all the surveyed locations in the field by counting number of plants infected using the formula given below.

$$\text{Per cent disease incidence} = \frac{\text{No. of plants infected}}{\text{Total no. of plants observed}} \times 100$$

Observations were also recorded on type of symptoms, total area, stage of the crop, variety grown and type of insects feeding.

3.2 SYMPTOMATOLOGY STUDY

To know the type of symptoms produced by banana bunchy top virus, the variety Robusta was inoculated by using banana aphid *Pentolonia nigronervosa* Coq. Young banana seedlings planted in pots were used for the above study. Aphids maintained on healthy banana were first starved for 15 minutes and then allowed for acquisition feeding

of 24 hours on diseased plants. After acquisition of the virus 20 viruliferous aphids were collected and released on to each of the healthy test banana plants. Inoculation feeding period of 8 hr was given to aphids on the test plants and then the plants were sprayed with 0.05 per cent Dimethoate (Rogar) to kill the aphids. The plants were labeled and were observed for one year for symptom expression in insect proof glasshouse. Observations were recorded on time taken for symptom expression, type of symptoms and severity of symptoms. Transmission was confirmed by ELISA.

3.3 TRANSMISSION STUDIES

3.3.1 Mechanical transmission

The mechanical transmission study was carried out using standard sap transmission method. Leaves showing typical bunchy top symptoms were collected from the plants maintained in insect proof glasshouse. The collected leaf samples were washed under tap water to remove any dust and soil adhered on it and dried on blotter paper. The sample was ground in 0.1 M potassium phosphate buffer pH 7.4 (containing 0.2 per cent (v/v) 2-Mercaptoethanol and 0.1 per cent (w/v) sodium diethyldithio carbamate) at the rate of 1 g of plant tissue in 2 ml of buffer using mortar and pestle. The extract was filtered through double layer muslin cloth and a pinch of Celite powder was added to the filtrate as an abrasive. A small amount of absorbent cotton was folded in the form of a pad, dipped in filtrate and rubbed gently, uniformly, unidirectionaly (along the parallel veins) on the upper surface of the leaves supported by a piece of

cardboard under lower surface to ensure uniform pressure and avoid leaf injury. The inoculated leaves were washed immediately with a jet of sterile water to remove the traces of celite. The plants were labeled and observed for symptom development up to one year in insect proof glasshouse. A total of ten plants were incubated. Observations on time taken for symptom expression, type of symptoms and severity of symptoms were recorded. The plants not showing any symptom were subjected to ELISA for confirmation of transmission

3.3.2 Vector (insect) transmission

Insect transmission studies were undertaken using three aphid species collected from different sources and maintained in insect cages.

Aphid species used	Source	Maintained on
1. *Aphis gossypii* Glover.	Cotton	Cotton
2. *Myzus persicae* Sulz	Radish	Chilli
3. *Pentolonia nigronervosa* Coq	Banana	Banana

These aphid species were identified based on following characteristics.

1. *Aphis gossypii* Glover : The greenish or light pink colored aphid occurs in large numbers on under surface of the leaves (Body length is 1.2 to 2.3 mm) (Plate 1).
2. *Myzus persicae* Sulz : Colour is varying from whitish or pale yellowish green to mild green (Plate 2).
3. *Pentalonia nigronervosa* Coq : The dark brown aphid occurs in colonies at axils of leaves (Plate 3).

Plate 1. *Aphis gossypii*.

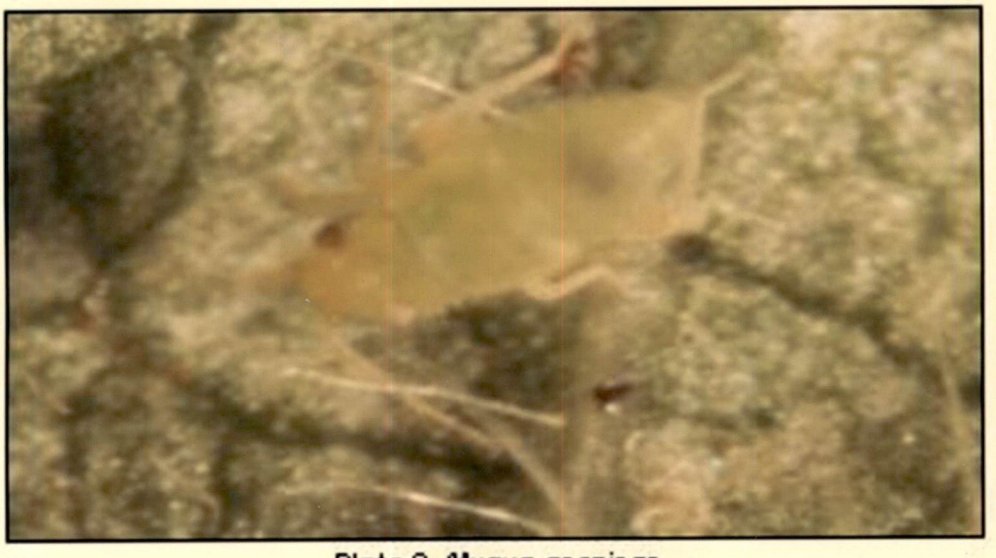


Plate 2. *Myzus persicae*.

Plate 3. *Pentalonia nigronervosa*.

Aphids of all the three species were first starved for 15 min, in a Petridish separately. These pre-starved aphids were gently transferred individually on to a diseased banana plants maintained in glasshouse, with the help of a fine camel hairbrush. The aphids were allowed for acquisition feeding for 24 hr. At the end of required feeding period 20 aphids were transferred on to each test plants. Inoculation feeding period of 8 hr was given to aphids on the test plants and then the plants were sprayed with 0.05 per cent Dimethoate (Rogar) to kill the aphids. Ten plants were inoculated by each aphids. The inoculated plants were labeled and kept in glasshouse for observation up to one year. Observations on time taken for symptom expression, type of symptoms and severity of symptoms were recorded. The inoculated plants were also subjected to ELISA for confirmation of transmission.

3.3.3 Sucker transmission

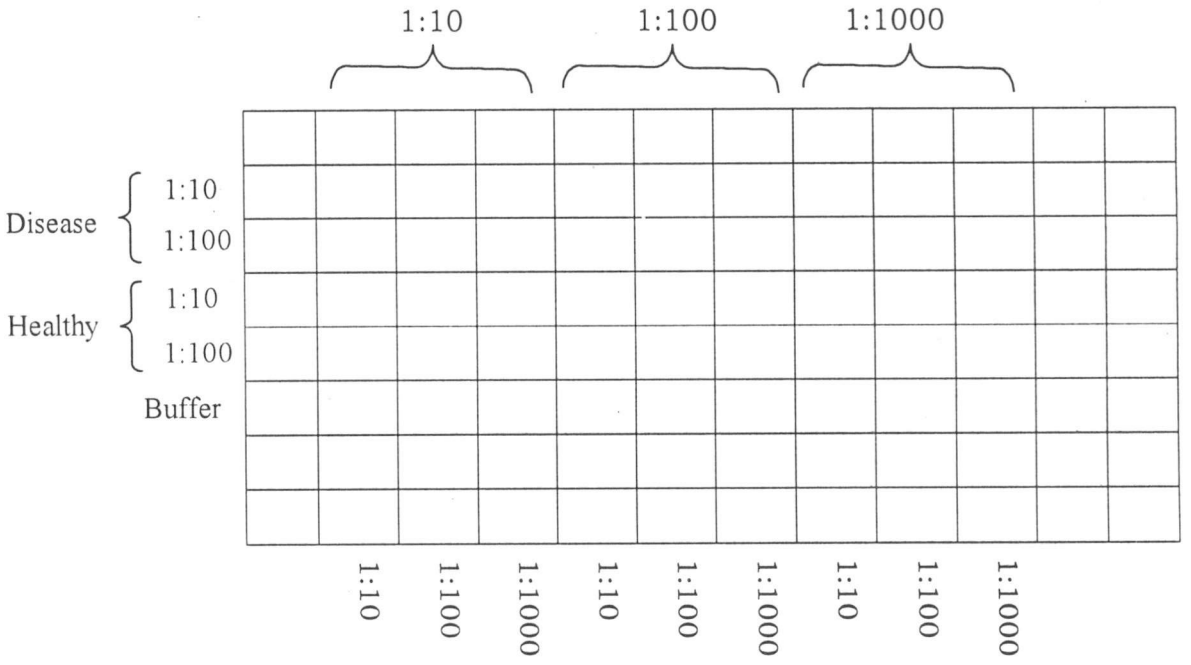
The suckers were collected from the field plants showing distinct symptoms of bunchy top disease and also from healthy plants. These were planted in earthen pots separately and maintained in insect proof glasshouse for symptom expression. Observations on time taken for symptom expression, type of symptoms and severity of symptoms were recorded during the growth of the plants. At the end of experimental period the transmitted plants were tested by ELISA for confirmation of transmission.

3.4 SERODIAGNOSTIC TECHNIQUE

3.4.1 Standardization of DAS-ELISA

The Antirabbit antibodies raised against BBTV and homologous-labeled antibodies conjugated with Alkaline phosphatase purchased from

Suyog Diagnostics Pvt. Ltd., New Delhi was used for Dooble Antibody Sandwich - Enzyme Linked Immuno Sorbent Assay. To know the optimum conditions for effective diagnosis of BBTV in samples the test was standardized as per the following plan.



Conjugate (Labeled antibody) dilutions

PLAN FOR STANDARDIZATION OF DAS-ELISA

Development of yellow colour was rated as positive as against no colour for negative samples. Further the intensity of colour was read in ELISA reader at 405 nm.

3.4.2 Detection of BBTV by DAS-ELISA

The detection of virus was carried out using DAS ELISA. All the following test samples were subjected for the above test.

S_1: Field sample with distinct symptoms

S_2: Artificial inoculated samples with symptoms

S_3: Mechanically inoculated sample

S_4: Sample inoculated by *Pentaloina nigronervosa*

S_5: Sample inoculated by *Myzus persicae*

S_6: Sample inoculated by *Aphis gossypii*

S_7: Sample from plants raised from infected suckers

S_8: Sample from tissue culture seedling raised from meristem of diseased mother plant.

S_9: Sample from tissue culture seedling raised from meristem of healthy mother plant.

S_{10}: Samples from healthy plants

Reagents

A. Coating / sample buffer (sodium carbonate buffer) pH 9.6

1.59 g Na_2CO_3

2.93 g $NaHCO_3$

0.2 g NaN_3

0.02 m DIECA

Dissolved in distilled water and volume was made to 1 liter.

B. Phosphate buffer saline (PBS) pH 7.4 (Stock buffer)

8 g $NaCl_2$

0.2 g KH_2PO_4

1.4 g Na_2HPO_4 $2H_2O$

0.2 g KCL

0.2 g NaN_3 dissolved in 1 liter distilled water.

C. Wash buffer (PBS-T)

Phosphate buffer containing 0.5 ml Tween 20 per liter.

Antibody buffer / sample buffer

PBS-T containing 2 per cent PVP (Polyvinyl pyriolodine)

Conjugate buffer

PBS-T containing 2 per cent PVP and 0.2 per cent ovalbumin

Substrate buffer

97 ml Diethanolamine dissolved in 800 ml distilled water and pH adjusted to 9.8 with 6 N HCl and volume made to 1 liter.

Blocking buffer

PBS-T containing 2 per cent PVP and 4 per cent skimmed milk powder.

Procedure

- 200 µl of coating buffer containing anti BBTV Ƴ globulin diluted to 1:100 was added in each well.
- Incubated at 37°C for 2 hours.
- Plate was washed with wash buffer thrice at three minutes interval.
- 200 µl of sample including buffer, healthy and positive control diluted to 1:10 in sample buffer was added in each well.
- Incubated at 37°C for 2.5 hours.
- Plate was washed with wash buffer (PBST) thrice at three minutes interval.
- 200 µl of conjugate buffer containing conjugated antibodies diluted to 1:100 was added in each well.
- Incubated at 37°C for 2.5 hours.
- Plate was washed with wash buffer (PBST) thrice at three minutes interval.
- 200 µl of freshly prepared substrate (P-nitrophenyl phasphate) was added in each well.
- Incubated at room temperature, observed for the development of colour.
- Reaction was stopped by adding 50 µl of 3 M sodium hydroxide solution in each well.
- Plate was red in ELISA reader (ELX 800 MS) at a wavelength of 405 nm.

Reactions were tabulated.

3.5 PRODUCTION OF DISEASE FREE PLANTINGS USING TISSUE CULTURE TECHNIQUE

Novel mesristem culture technique was adopted for Production of healthy plants from diseased meristems. The diseased suckers of Cavendish variety were collected from the field and used for the study along with healthy suckers as control. All the micropropgation activities were carried out at tissue culture laboratory, Department of Horticulture, UAS, Dharwad.

3.5.1 Preparation of explants

Suckers collected from the field were cleaned and washed with water. The corms were trimmed by removing outer leaves and corm tissue were trimmed carefully using knife at the size of 4 to 5cm long and 1cm diameter. The meristem buds were washed using Tween-20 to remove the adhering soil particles followed by three times washing with ordinary water. Then the buds were sterilized with 0.1 per cent Bavistin solution for 3 minutes and followed by three washings with sterile double distilled water. The buds were next surface sterilized with 70 per cent alcohol for 30 seconds followed by three washings with sterile double distilled water. The later two surface sterilizations were done under Laminar Air Flow Chamber (LFC).

3.5.2 Initiation of culture

Outer leaf sheaths from the meristem buds were removed until meristem retains one or two layers of leaf sheath along with small base in a petriplate containing small amount of 0.1 per cent ascorbic acid. The meristems were then inoculated on B_1 medium (MS basal supplemented with 2 mg/l Benzyl adenine) and kept for shoot proliferation at 23±2^{0}C

with 16 hr photoperiod. The cultures were subcultured whenever browning of medium was observed due to exudation of phenolics.

3.5.3 Multiplication of plantlets

After proliferation of shoots they were cut longitudinally and inoculated for multiplication on B_2 medium (MS basal supplemented with 5 mg/l Benzyladenine and 30-mg/l-adenine sulphate along with 0.8 per cent agar as a solidifying agent) for shoot multiplication. Subculturing was done at weekly intervals.

3.5.4 *In vitro* rooting of shoots

Multiple shoots formed were separated individually and inoculated in B-3 rooting medium (MS basal supplemented with 3 per cent sucrose, 0.8 per cent agar as solidifying agent and supplemented with NAA 1 mg/l). The cultures were incubated for a period of twenty days. The rooted plantlets were transferred to hardening and those non-rooted were again subcultured to the same medium for another 15 days.

3.5.5 Hardening of plantlets

The rooted plantlets were taken out from the bottles after rooting and washed in water to remove any adhered medium on the basal portion. The plantlets were kept immersed in water for 12 hours and later treated with 0.1 per cent bavistin solution for one hour and finally rinsed in tap water. The plantlets were then transferred to net pots containing sterilized sand and placed in green house for a period of one week, after which they were transferred to polybags containing potting media (Soil, Sand, Compost, Coir Dust in 1:1:1:1 ratio) without damaging the root system and kept under the green house for another one month.

EXPERIMENTAL RESULTS

IV. EXPERIMENTAL RESULTS

The results of the investigation carried during the period of 2003-04 on the Banana bunchy top disease are presented here.

4.1 SURVEY FOR DISEASE INCIDENCE

Roving survey to know the incidence of Banana Bunchy Top Virus (BBTV) disease was carried out in three districts *viz.*, Belgaum, Haveri, and Dharwad. A total of 39 villages, 14 from Belgaum, 9 from Dharwad and 16 from Haveri were surveyed.

The data on survey in Belgaum district, presented in Table 1 revealed presence of Bunchy Top disease in seven villages out of fourteen villages surveyed. The incidence ranged from 0.09to 11.11 per cent. The highest incidence of 11.11 per cent was recorded in Itagi village followed by 6.94 per cent in Kadaravalli village both belonging to Bailhongal taluk. The lowest incidence of 0.09 per cent was found in Hirebagewadi village of Belgaum taluk. Sondargi in Bailahongal Taluk, Khanapur and Ramapur in Khanapur Taluk, Sutagatti in Belgaum Taluk, Hooli and Soundatti in Soundatti Taluk were free from disease.

In Dharwad taluk except Dharwad (UAS Campus) the disease was present in all the villages surveyed (Table. 2). The disease ranged from a minimum of 0.02 per cent in Hubli (Hubli taluk) with Robusta variety to maximum of 3.47 per cent at Gambyapur village (Khalghatgi Taluk) with same variety. Overall incidence of the disease in the district was low.

Table 1. Survey of Banana Bunchy Top Disease at Belgaum District.

Sl. No.	Taluk	Place	Area (Acre)	No. of diseased plants	Disease incidence (Percentage)	Variety planted	Stage of crop (months old)	Vector found	Symptoms observed
1	Bailhongal	M. K. Hubbli	5	0	0	Robusta	16	-	-
		Kadaravalli	2	250	6.94	G-9	5	Ap.C	SG, DGSL NLTl, D
		Itagi	2	400	11.11	G-9	18	Ap.C	SG, DGSL, NF, D
		Kittur	3	50	0.92	G-9	5	Ap.C	SG, VC
		Sondargi	2.5	0	0	Shrimanti	8	-	-
2	Khanapur	Khanapur	1.5	0	0	G-9	15	-	-
		Ramapur	2	0	0	Robusta	9	-	-
3	Belgaum	Kuruvinakaoppa	2	80	2.2	Robusta	5	Ap.C	SG, DGSL, MC
		HireBagevadi	3	5	0.09	Robusta	12	Ap.C	SG, DGSL
		Sutagatti	1.5	0	0	G-9	5	-	-
4	Soundatti	Hooli	3	0	0	Robusta	5	-	-
		Soundatti	2.5	0	0	Local	24	-	-
		Munavalli	1	50	2.7	Robusta	16	Ap.C	SG, DGSL
		Gokak	2	150	4.16	Rajapuri	12	Ap.C	SG, DGSL, VC
				Average	2.00				

Where - = Absent
DGSL=Dark green streak on leaf lamina,
TL-Thickening of leaf lamina,
Ap.C=Aphid colony,
NF=Non flowering of plant,
VC-Vein Clearing, MC-Marginal chlorosis
SG=Stunted growth,
NL-Narrowing of leaf lamina,
D-Death of plant.

Table 2. Survey of Banana Bunchy Top Disease at Dharwad District.

Sl. No.	Taluk	Place	Area (Acre)	No. of diseased plants	Disease incidence (Percentage)	Variety planted	Stage of crop (months old)	Vector found	Symptoms observed
1	Dharwad	UAS campus	1	0	0	Robusta	12	-	-
		Nuggikeri	3	5	0.09	G-9	8	-	SG, DGSL, VC & MC
2	Hubli	Aralikatti	2	15	0.41	Robusta	11	-	SG
		Chabbi	1.5	12	0.44	Robusta	16	Ap	BF, NF, NL & TL
		Noolvi	1	5	0.27	G-9	4	-	SG & DGSL
		Hubli	5	2	0.02	Robusta	15	-	SG
3	Khalaghatgi	Gambyapur	4	250	3.47	Robusta	8	Ap	SG
		Hirehonnalli	2	55	1.52	Robusta	14	Ap	SG
		Dastikoppa	3.5	5	0.07	Robusta	9	-	SG
				Average	0.69				

Where
- = Absent
Ap.= Aphid , SG = Stunted growth, DGSL=Dark green streak on leaf lamina, NF=Non flowering of plant,
NL=Narrowing of leaf lamina, TL=Thickening of leaf lamina, VC=Vein Clearing, BF=Bouquet formation and
D=Death of plant.

In Haveri district Bunchy Top incidence was recorded in ten villages out of sixteen villages surveyed. It is evident from the survey results presented in Table 3 that highest incidence of 2.44 per cent was noticed in an orchard with Robusta variety at Savanur (Savanur Taluk) followed by 2.08 per cent at Rattihalli of Hirekerur Taluk again with Robusta variety. A least incidence of 0.14 per cent was recorded in Dundasi village of Shiggaon Taluk with G-9 cultivar. Like Dharwad district overall incidence was low in Haveri district also.

In general the disease incidence ranged from 0.02 per cent to 11.11 per cent. Incidence of bunchy top was high in Belgaum district followed by in Dharwad (0.69 %) and Haveri (0.56 %) district (Table 1, 2 and 3).

Among the different cultivars grown in different districts an average the disease incidence was more in orchards with Rajpuri cultivar (4.16%) followed by G-9 (1.88%) and Robusta (0.77%). But cultivar G-9 was the most susceptible with a maximum incidence of 11.11 per cent (Table 4). The orchards planted with Shrimanti and Munavalli local were totally free from bunchy top incidence. Variety Robusta was the most popular one, which was seen in 24 fields followed by G-9 in 11 fields.

The disease was noticed at all stages of crop growth. Symptoms were more severe on early-infested plants. The symptoms observed during the survey were dark green streaks on the leaf lamina (Plate 4), pale yellow leaves with ragged margins (Plate 5) irregular leaf blade development and vein clearing (Plate 6), progressive reduction of leaf

Table 3. Survey of Banana Bunchy Top Disease at Haveri District.

Sl. No.	Taluk	Place	Area (Acre)	No. of Diseased plants	Disease Incidence (Percentage)	Variety planted	Stage of crop (months old)	Vector found	Symptoms observed
1	Haveri	Ekkari	1	0	0	Robusta	15	-	-
		Hosaritti	4	40	0.5	Robusta	5	-	SG & DGSL
		Chennur	4	55	0.76	G-9	4	AP.C	SG, NL, TL & BF
		Akkur	10	0	0	Robusta	5	-	-
		Guttala	2	0	0	G-9	14	-	-
2	Savanur	Savanur	2	88	2.44	Robusta	12	AP.C	SG & BF
		Dombarmuttar	0.5	5	0.5	G-9	10	-	SG, BF & DGSL
		Hattimattur	3	0	0	Robusta	15	-	-
3	Shiggaon	Shiggaon	2	0	0	Robusta	5	-	-
		Dundasi	3	8	0.14	G-9	11	-	SG, BF, DGSL, VC & TL
4	Hirekerur	Rattihalli	2	75	2.08	Robusta	10	-	SG, BF & NL
		Masoor	1.5	41	1.51	Robusta	6	-	SG, BF, NL & DGSL
		Koda	3	10	0.18	Robusta	9		SG & BF
5	Ranebennur	Haranahalli	2	0	0	Local	8	-	-
		Makanoor	0.75	8	0.61	Robusta	12	-	SG, BF, NL & VC
		Kavalettu	1.5	7	0.25	Robusta	10	-	SG, BF
				Average	0.56				

Where:
- = Absent
NF-Non flowering of plant
BF-Bouquet formation
Ap.C=Aphid colony
NL-Narrowing of leaf lamina
D-Death of plant.
SG-Stunted growth ,
TL-Thickening of leaf lamina
DGSL-Dark green streak on leaf lamina,
VC-Vein Clearing ,

Table 4. Varietal reaction to incidence.

Sl. No.	Cultivar	No. of fields	Range	Average PDI
1	Robusta	24	0.02 to 3.47	0.77
2	G-9	11	0.09 to 11.11	1.88
3	Shrimanti	1	0	0
4	Local Munavalli	2	0	0
5	Rajapuri	1	4.16	4.16

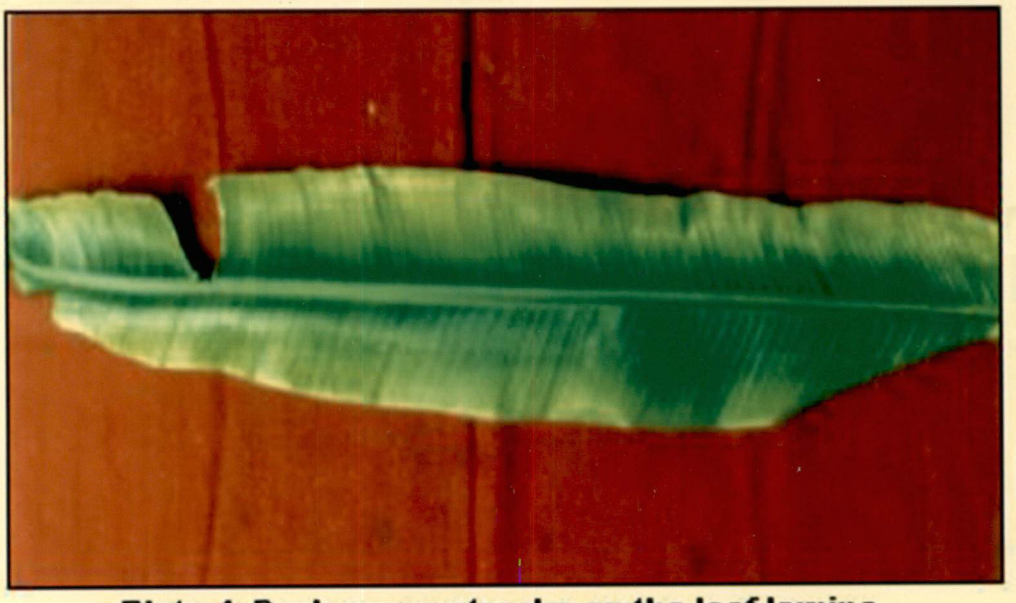


Plate 4. Dark green streaks on the leaf lamina.

Plate 5. Pale leaves with ragged margins .

Plate 6. Irregular leaf blade development and vein clearing

lamina size in younger leaves (Plate 7), marginal Chlorosis (Plate 8), narrowing of the leaf lamina (Plate 9), thickening leaf blade (Plate 10) reduction in petiole length (Plate 11), upright leaves (Plate 12) bouquet formation (Plate 13), broom head appearance of the foliage(Plate 14), malformation of the entire plant (Plate 15), stunting of plant (Plate 16), non-flowering of the plant (plate 17), finally death of the plants were observed (Plate 18) and view of abandoned banana orchard (Plate 19).

Banana aphids (*Pentalonia nigronervosa* Coq) were found feeding or colonising in some orchards but it was not a general feature. Other than aphids no insects were found in any of the orchards surveyed.

4.2 SYMPTOMATOLOGY

The Symptomatalogical studies were carried out by using standard insect transmission methodology. The banana plants inoculated by Bunchy Top virus with the help of aphid *P. nigronervosa* developed the symptoms after six months of inoculation. The inoculated plants remained stunted with erect and brittle dark green leaf lamina. The leaves in these infected plants were narrow (Plate 20).

4.3 TRANSMISSION STUDIES

4.3.1 Mechanical transmission

The mechanical transmission was carried out using sap inoculation method on Robusta cultivar of banana plants. The results (Table 5) indicated that the virus under study was not sap transmissible as none of the sap inoculated plants developed symptoms even up to one year. These plants tested negative even in ELISA.

Plate 7. Progressive reduction of leaf lamina in younger leaves.

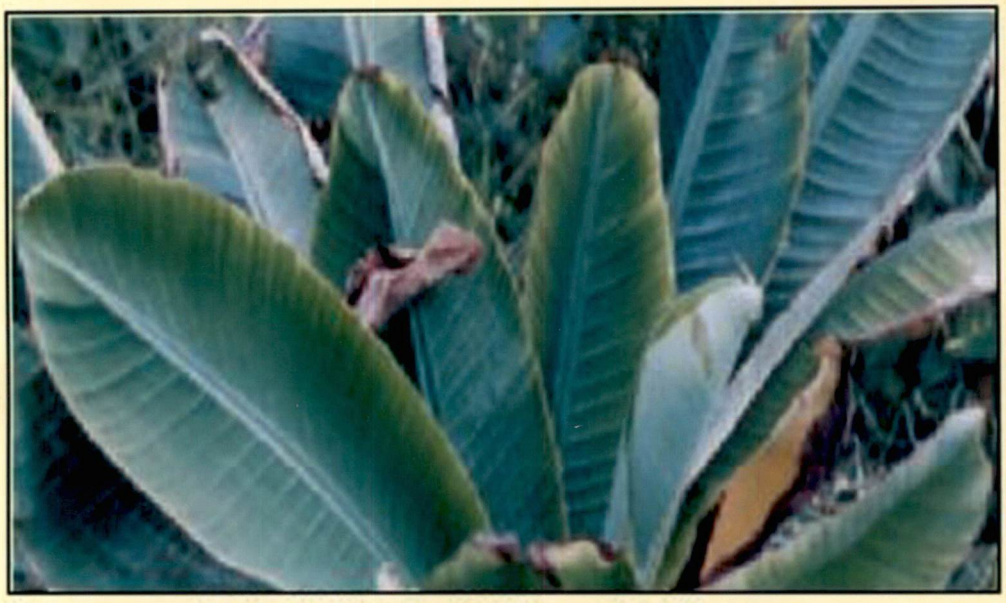


Plate 8. Marginal chlorosis of leaves.

Plate 9. Narrowing of the leaf lamia.

Plate 10. Thickening of the leaf blade in infected plant.

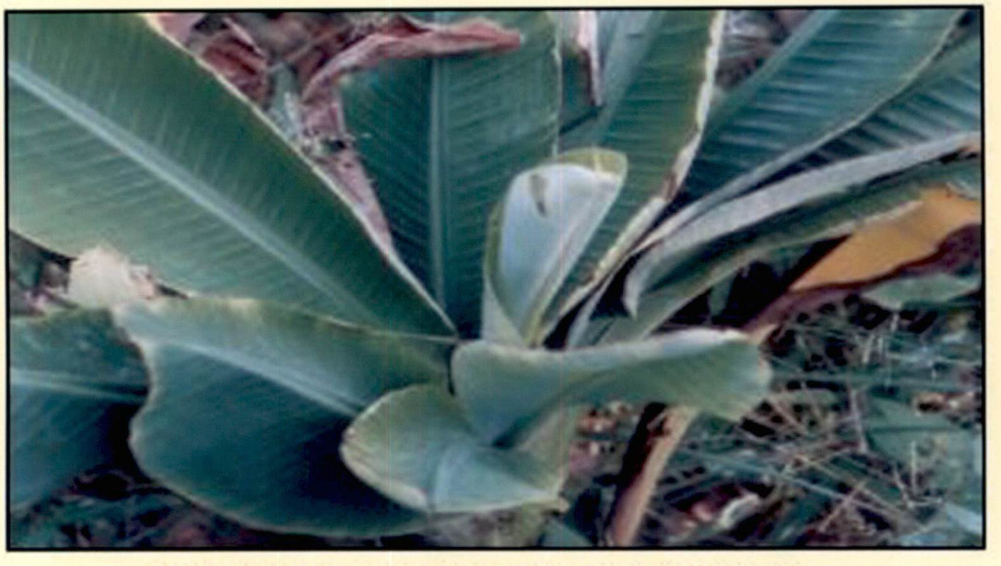


Plate 11. Reduction in petiole length of leaves.

Plate 12. Upright leaves on infected plant.

Plate 13. Bouquet formation of entire plant.

Plate 14. Broom like appearance of infected plant.

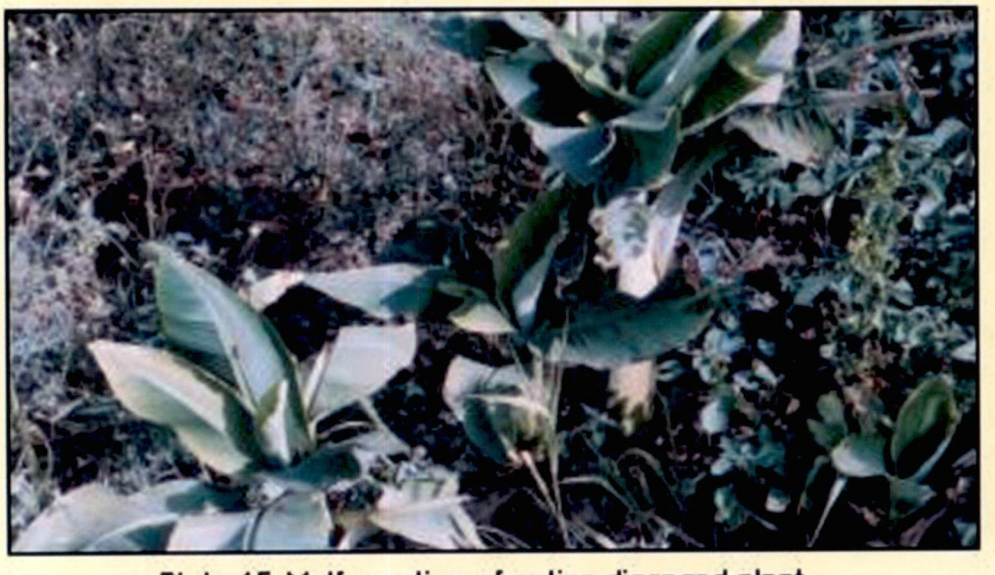


Plate 15. Malformation of entire diseased plant.

Plate 16. Stunting of diseased plant.

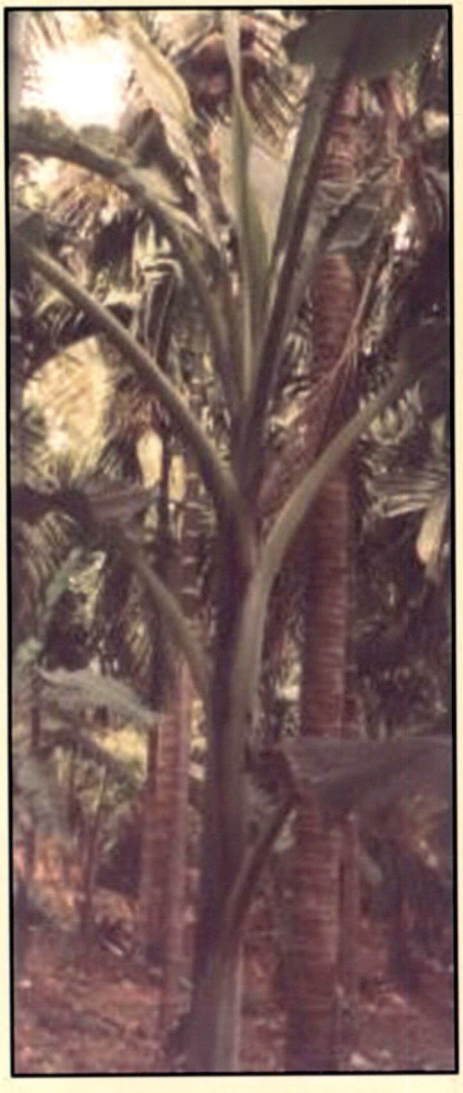


Plate 17. Non flowering of the diseased plant.

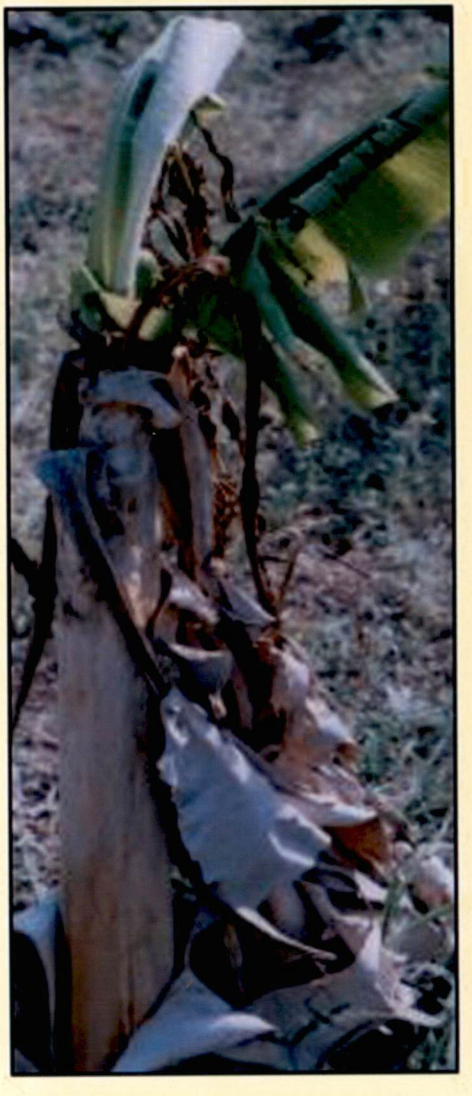


Plate 18. Death of infected plant.

Plate 19. View of abandoned banana orchard.

4.3.2 Vector (Insect) transmission

Three aphid species *viz.*, *Pentalonia nigronervosa*, *Myzus persicae* and *Aphis gossypii* were used for vector transmission studies as described in material and methods. It is evident from the data presented in Table 5 that only *P. nigronervosa* successfully transmitted BBTV. Seven out of ten inoculated plants developed symptoms like stunted growth, dark green leaves and bouquet formation of foliage, six months after inoculation. The other two aphids failed to transmit the virus as none of the ten plants each inoculated by *Myzus persicae* or *Aphis gossypii* developed any symptoms during the entire course of investigation. The transmission of Bunchy Top virus by *Pentalonia nigronervosa* was further confirmed by ELISA.

4.3.3 Sucker transmission

Suckers collected from BBTV infected plants were planted in pots and kept in glasshouse for symptom expression and the results are presented in Table 5. After four months all ten plants grown from infected suckers developed very severe symptoms of BBTV *viz.* severe stunted growth, narrowing of leaf lamina, reduced petiole length, dark green streaks on leaf lamina (Plate 21 and 22). The results were further confirmed by ELISA.

4.4 SERODIAGNOSIS

4.4.1 Standardization of DAS-ELISA

A Double Antibody Sandwitch Enzyme Linked Immuno Sorbant Assay (DAS-ELISA) was standardized to know the optimum conditions

Table 5. Transmission studies of BBTV by different methods of transmission.

Sl. No.	Methods of transmission	No of plants infected/ Inoculated	Percentage transmission	Time taken for symptom expression
1	Mechanical	0/10	0	No symptoms
2	Vector			
	a) *Pentalonia nigronervosa*	7/10	70	6 months
	b) *Myzus persicae*	0/10	0	No symptoms
	c) Aphis gossypii	0/10	0	No symptoms
3	Infected suckers	10/10	100	4 months

Plate 20. Vector *(Pentalonia nigronervosa)* transmitted plant showing symptoms of BBTV.

Plate 21. Sucker transmitted plant showing BBTV symptoms.

Plate 22. Sucker transmitted plant showing BBTV symptoms.

for quick and effective detection of BBTV as described in material and methods. The results are presented in Table 6. On visual observation it was noticed that the yellow colour developed in wells loaded with disease sample diluted to 1:10 and 1:100, coating antibodies diluted up to 1:100 and conjugate diluted up to 1:100. The wells containing further diluted reagents and also healthy samples as well as buffer were colorless. Similar results were observed when the plate was red at 405 nm in ELISA reader.

Highest OD values of more than 0.390 were recorded when the sample as well as coating antibodies were diluted to 1:10. The values reduced on further dilution of coating antibodies. The optimum OD of 0.386 was recorded when the sample was diluted to 1:10, coating and conjugated antibodies to 1:100.

4.4.2 Detection of virus by DAS-ELISA

For detection of virus in different samples collected from field, different experiments and tissue culture seedlings, DAS-ELISA was employed. The data presented in Table 7 indicated presence of virus in field collected diseased samples, samples inoculated by *Pentalonia nigronervosa*, samples from seedlings raised from diseased suckers and tissue culture seedlings raised from meristem of diseased mother plants. All the plants which tested positive in ELISA had clear cut bunchy top symptoms except tissue culture seedlings which raised from meristem of diseased mother plant. It was evident that the meristem tissue culture technique failed to eliminate virus. But these plants carried virus

Table 6. Standardization of DAS-ELISA for detection of BBTV.

Sample	Dilutions	Absorbance values at 405 nm								
		Coating antibody dilutions								
		1:10			1:100			1:1000		
		Conjugate dilutions								
		10^{-1}	10^{-2}	10^{-3}	10^{-1}	10^{-2}	10^{-3}	10^{-1}	10^{-2}	10^{-3}
Disease	1:10	0.395	0.390	0.391	0.387	0.386	0.110	0.090	0.052	0.005
Disease	1:100	0.285	0.256	0.210	0.208	0.120	0.092	0.040	0.006	0.004
Healthy	1:10	0.008	0.008	0.009	0.009	0.006	0.005	0.006	0.004	0.003
Healthy	1:100	0.006	0.004	0.008	0.002	0.004	0.006	0.006	0.003	0.003
Buffer	-	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 7. Serodiagnostic test (DAS-ELISA) of different samples.

Sl. No.	Samples from	Symptoms	Reaction
1	S_1	+	+
2	S_2	+	+
3	S_3	-	-
4	S_4	+	+
5	S_5	-	-
6	S_6	-	-
7	S_7	+	+
8	S_8	-	+
9	S_9	-	-
10	S_{10}	-	-

Where
+ Present
- Absent
S_1: Field sample with distinct symptoms
S_2: Artificial inoculated samples with symptoms
S_3: Mechanically inoculated sample
S_4: Sample inoculated by *Pentaloina nigronervosa*
S_5: Sample inoculated by *Myzus persicae*
S_6: Sample inoculated by *Aphis gossypii*
S_7: Sample from plants raised from infected suckers
S_8: Sample from tissue culture seedling raised from meristem of diseased mother plant.
S_9: Sample from tissue culture seedling raised from meristem of healthy mother plant.
S_{10}: Samples from healthy plants

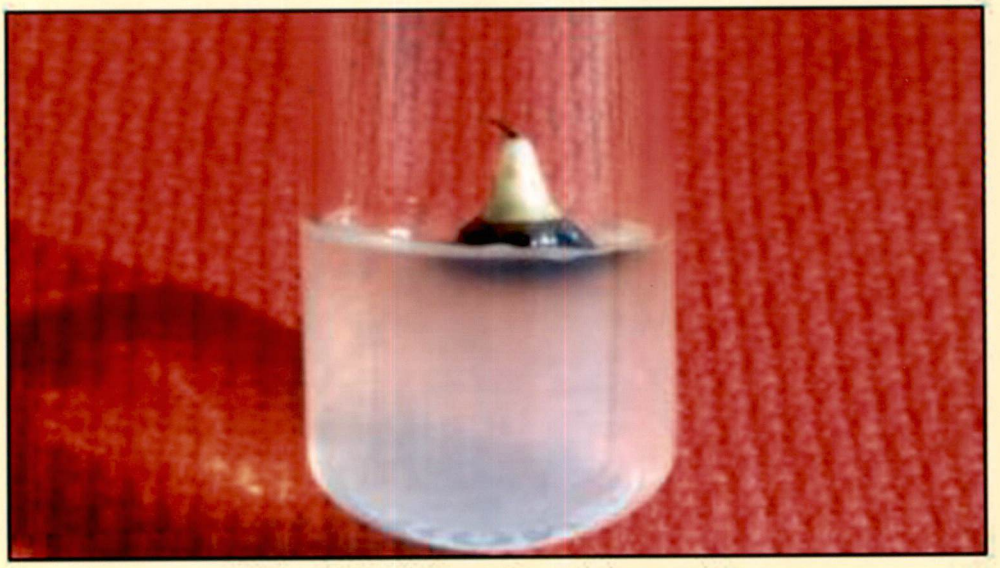


Plate 23. Initiation of meristem culture.

Plate 24. Proliferation of shoots.

Plate 25. Multiplication of shoots.

without any external symptoms. The rest of the samples (S_3, S_5, S_6, S_7 and S_{10}) were negative in ELISA.

4.5 PRODUCTION OF DISEASE FREE PLANTLETS USING TISSUE CULTURE TECHNIQUE

Meristem culture technique was employed for production of healthy plants. MS media as a basal medium supplemented with different growth regulators was used for different stages of meristem culture regeneration.

4.5.1 Preparation of explants

Suckers collected from infected plants from field were used for meristem culture. The trimmed, washed and sterilized meristem buds of 4 to 5 cm length were used for excising meristem of the explant.

4.5.2 Initiation of culture

Meristems excised (Plate 23) from the buds were inoculated aseptically under LFC on B-1 medium for initiation. The regenerated meristems were subcultured on the same medium on 10 days interval till shoot proliferation. Total 35 days were taken for shoot proliferation (Plate 24).

4.5.3 Multiplication of plantlets

The shoot prolifered meristems were cut longitudinally and inoculated for multiplication in B-2 medium. After 40 days multiple shoots were observed and were less in number compared to healthy shoot multiplication. The multiplied shoots were subjected for second cycle of multiplication, where the results were found non consistent (Plate 25).

4.5.4 *In vitro* rooting of shoots

Multiplied shoots were separated in single plantlets and inoculated on B-3 rooting medium. The root induction was observed after 25 days of inoculation. Some of the shoots, unabled to root were subcultured for once and found rooted after another 15 days.

4.5.5 Hardening of plantlets

The rooted plantlets were taken out from the bottles and washed to remove the adhered media. The plantlets were kept immersed in water at room temperature for 12 hours, treated with 0.1% bavistin solution for 1 hr and finally rinsed in tap water. Later the plantlets were planted in hardening plastic cups containing sterile sand and kept in green house for further hardening. The tissue culture seedlings raised from infected meristem or healthy meristem did not develop any symptoms even during the entire course of investigation (Plate 26). However, the seedlings raised from infested meristem tissue carried virus, which was confirmed by ELISA.

Plate 26. Different stages of hardening.

DISCUSSION

V. DISCUSSION

The viral diseases are one of the major constraints in banana cultivation. After sigatoka disease, banana bunchy top disease is the second largest damaging factor among the diseases occurring on banana. The disease was known to cause 60 to 100 per cent yield loss in severely infected orchards. Very rare information is found about the disease in Karnataka even though the disease is existing since many years. Considering this aspect the present investigation was undertaken to know the incidence level of disease in the fields, its symptomatalogy and management by meristem culture technique along with transmission studies.

A roving survey carried out to know the incidence of Banana Bunchy Top Virus (BBTV) in three districts *viz.* Belgaum, Dharwad and Haveri revealed presence of disease in all the districts. However the over all incidences was more in Belgaum district, where incidence ranged from 0.09 to 11.11 per cent. The disease was recorded in seven villages out of fourteen surveyed. Though incidence was low ranging from 0.02 to 3.47 in Dharwad district, the disease was recorded in most of the villages except Dharwad. Similarly low incidence ranging from 0.14 to 2.08 per cent of bunchy top was recorded in ten orchards out of sixteen surveyed in Haveri district.

Similarly Thiribhuvanamala and Doraiswamy (2001) during the survey noticed highest incidence (26.4 %) of Bunchy Top disease in Thadiyankudisai in Dindigal district and least in Coimbatore, Namakkal,

Theni, Tanjore and Erode districts with range from 0.86 to 2.20 per cent. There was no incidence of Bunchy Top in Trichy district.

Govindaswamy *et al.* (1977) observed that the incidence of Bunchy Top ranging from 10 to 40 per cent in ten villages of lower Pulney hills and maximum of 80 per cent in Agamalai area.

Among different cultivars grown in three districts, variety Robusta was the most popular one, which was seen in 24 fields followed by G-9 in 11 fields. Disease incidence was low in orchards planted with Robusta cultivar (0.77 %) whereas highest incidence of the disease was recorded in fields grown with G-9 cultivar. Similarly Selvarajan (1998) also reported 0 to 60 per cent Banana Bract Mosaic Virus infection in Nendran cultivar in Trichy district and 0.9 to 24.5 per cent Banana Streak Virus infection in Poovan variety. Mohan and Lakshmanan (1988) also found bunchy top disease incidence on all the popular varieties of the banana in Tamil Nadu.

Disease was observed at all stages of crop growth and exhibited various type of symptoms like dark green streaks on the leaf lamina, irregular leaf blade development and vein clearing, marginal chlorosis, reduction in petiole length, progressive reduction of leaf lamina size in younger leaves, bouquet formation, stunting of plant, thickening of leaf blade, malformation of the entire plant, narrowing of the leaf lamina, broom head appearance of the foliage, non-flowering of the plant, leaves with pale yellow colour having the ragged margins, upright leaves and finally death.

Similarly Vakili (1969) observed progressive reduction in leaf lamina and reduction in petiole length in plants affected by bunchy top disease. Khalid and Soomro (1993) also observed similar type of symptoms on samples collected from diseased plants, which had short, erect leaves, severe stunting and pronounced bunchy top appearance. Further the disease recognized by bouquet formation, stunting of plant, thickened leaf blade, narrowing of the leaf lamina, broom head appearance of the foliage. Similarly Alagiamanavalan *et al.* (1973) observed highly stunted growth with severe reduction in leaf size, chlorotic, brittle and upright leaves in affected plants. The leaves appeared crowded at the throat region. Such plants bear no flower, and finally succumbed to death. Singh (1979) observed that affected plants show narrow chlorotic leaves with pale and ragged margins which emerge only partially thus forming a bunch at the apex of the plant.

Banana plants, which were artificially inoculated by banana aphids in glasshouse remained stunted with erect, brittle dark green leaf lamina six months after inoculation. Singh (1979) also recorded similar type of symptoms in artificially inoculated banana plants.

In the present study, the bunchy top virus under study was not transmitted by mechanical inoculation. All the ten sap inoculated plants remained unaffected and did not develop any symptoms even up to one year. These plants tested negative even in ELISA test. These results are in confirmation with the observation of Khurana (1967) who did mechanical transmission using sap from infected plants and reported

that bunchy top disease was not mechanically transmissible. Similarly Magee (1927), Raj (1970) and Singh (1979) failed to transmit the virus by mechanical inoculation.

Among three-aphid species *viz.*, *Pentalonia nigronervosa*, *Myzus persicae* and *Aphis gossypii* used for transmission of the virus, only *Pentalonia nigronervosa* was found capable of transmitting BBTV from diseased to healthy plants. The banana aphids successfully transmitted the virus after 20 minutes starvation, 24 hours acquisition access and 8 hours inoculation feeding period. Whereas *Myzus persicae* and *Aphis gossypii* failed to transmit the virus as none of the plants inoculated by these aphids developed symptoms, even up to one year and further tested negative in ELISA. In the field studies of Nair *et al.* (1973), banana aphid *Pentalonia nigronervosa* was the only aphid found responsible for transmission of the virus causing bunchy top disease in banana. Concurrence results were also recorded by Allen (1978), Singh (1979), Reghupathy (1980), Wu and Su (1990b), Drew et al.(1992), Mariappan and Mathikumar (1992), Thiribhuvanamala *et al.*,(2001).

The suckers collected from diseased plants started showing symptoms of BBTV after four months of planting. Such plants exhibited stunted growth, narrowing of leaf lamina, reduced petiole length, dark green streaks on leaf lamina. The symptoms were very severe probably because the infection was in earliest stage of growth of the plants. Similarly Mariappan and Mathikumar (1992) found BBTV transmission through infected suckers. Nodwora *et al.* (2000) also confirmed the

dissemination of BSV through suckers. Kenganal (2003) noticed 100 per cent transmission of BSV by vegetative propagation planting material in the field.

A DAS-ELISA protocol by using commercially produced antiserum and conjugate homologous to BBTV was standardized. The virus was detected in disease samples when the sample was diluted to 1:10, coating antibodies to 1:100 and conjugate up to 1:100. Even though virus could also be detected at lower dilutions of coating and conjugated antibodies but it was not economical. Virus was also detected even when the sample was diluted to 1:100, but if the concentration of virus in the sample is low, it may not work satisfactory. Hence DAS-ELISA test could effectively be employed at 1:10 dilution of sample, 1:100 dilution of both coating and conjugated antibodies as optimum conditions. Further dilutions of the reagents may not give satisfactory results. No colour development in healthy sample wells indicated that the antibodies were good and in buffer wells indicated that the test was satisfactory. Hence the test was successfully employed for testing presence of virus in ten different samples. The leaf samples collected from field plants with typical symptoms, from the plants inoculated by *P. nigronervosa* from plants grown with infected suckers and even the plantlets raised by meristem culture tested positive in the ELISA test. However, plants inoculated by sap, *Myzus persicae, Aphis gossypii* were free from the virus.

Several workers have employed ELISA for detection of virus and suggested ELISA as a highly sensitive test than any other methods and

further suggested that the test could be used for quick detection of virus was possible in infected plants even when the virus concentration was low Thomas and Dietzgen (1991), Drew *et al.* (1992), Mariappan and Mathikumar (1992), Khalid and Soomro (1993) and Singh and Verma (2002).

The meristem tissue culture technology has been employed for elimination of the virus in tissue culture seedlings on the consideration low concentration of virus, highly multiplying cells, presence of less number of plasmodesmata and meristematic cell constituents inhibitory for multiplication of virus. Hence attempts were made to know the elimination of the virus by meristem culture technology, which could be employed as a management strategy for bunchy top disease. The seedlings raised from the meristem excised from disease plants using three different media, prepared using MS (Murashige and Skoog, 1962) medium as a basal dose supplemented with different growth regulators for different stages. Plantlets after regeneration and multiplication were rooted in half strength MS medium using B3 medium. The rooted plants were subjected to hardening in green house by planting in sterile sand. Laxmikanth and Nataraja (1989) also successfully regenerated banana plantlets using meristem as explant. In the similar efforts by Wu and Su (1991) BBTV infected tissue were used for eliminating the virus in their study. The regenerated plantlets did not exhibit any symptoms even up to one year but carried banana bunchy top virus. Such of the plants tested positive in ELISA indicating, failure of meristem tissue culture technique in elimination of the BBTV. From these findings it is evident

that BBTV could not be eliminated by meristem culture technique. It may be because of the difficulty in getting the exact meristem which could be free from the virus owing to very small size (Selvarajan NRC on Banana, Trichy personal communication). Similar results were obtained by Drew *et al.* (1992) who observed very severe symptoms of bunchy top disease in all the regenerated plantlets through meristem tissue culture technique 12 months after hardening. However, those plantlets remained symptoms less in early stage under *in* vitro conditions. Further they concluded that while selecting the explant for commercial mass multiplication all the explants should be carefully monitored for symptoms by a well-experienced pathologists. Kenganal (2003) found that meristem culture alone or in combination with thermotherapy could not eliminate the BSV from the infected meristems hence in these types of viruses, indexing of (Carpio, 2002) virus in micropropagated material is best approach of control. Quarantine was also found as another well-suited control measure in international levels of germplasm and planting material exchanges.

Future line of work

1. Meristem culture technique has to be standardize for production of virus free plantlets from diseased mother plants.
2. Owing to difficulty in purification and production of quality antiserum, other diagnostic techniques have to be standardize.
3. Attempts have to be made for development of transgenic banana to manage the disease.

SUMMARY

VI. SUMMARY

Among viral diseases of Banana, Bunchy top is one of the major diseases causing greater economic loss. Survey revealed the presence of disease in all three districts *viz.* Belgaum, Dharwad and Haveri. The incidence ranged from 0.02 per cent to 11.11 per cent. Incidence was high in Belgaum district followed by Dharwad (0.69%) and Haveri (0.56%) district.

Among different cultivars, under cultivation in these districts, G-9 was more susceptible with incidence of 11.11 per cent followed by Rajpuri (4.16%). The most popular variety Robusta had comparatively less disease. The orchards planted with cultivar Munavalli and Shrimanti were free from Bunchy Top incidence.

The disease was noticed in all most all stages of crop growth. Symptoms were severe on early-infected plants. Dark green streaks on leaf lamina, thickening and narrowing of leaves, vein clearing, marginal chlorosis, irregular leaf blade development, reduction in petiole length, bouquet formation, stunted growth of plant, malformation of entire plant, leaves with pale yellow colour having the ragged margins, upright leaves and death of plants were the different symptoms observed during the survey.

The artificially inoculated plants of Robusta cultivars in glasshouse developed symptoms after 6 months. Such plants remained stunted and produced dark green, narrow, erect and brittle leaves.

The BBTV was not sap transmissible as none of the ten sap inoculated plants expressed symptoms even up to one year. Among the insect vectors, only *Pentalonia nigronervosa* transmitted virus. Other two

aphids *Myzus persicae* and *Aphis gossypii* failed to transmit the virus. Whereas virus was readily carried by suckers as all the ten plants raised from suckers developed bunchy top symptoms.

A DAS-ELISA technique was standardized using antibodies raised against BBTV and homologous conjugate procured commercially from Suyog Diagnostics Pvt. Ltd. New Delhi. The test detected the virus at 1:10 dilution of sample, 1:100 dilutions of antibody and conjugate. The samples, *viz.* field sample with distinct symptoms, plants inoculated by *Pentalonia nigronervosa* plants raised from disease suckers, plants raised from meristem excised from disease plants reacted positive to the ELISA test. Meristem tissue culture seedlings did not develop any symptoms even up to one year but were found positive in ELISA. Hence it is obligatory to use healthy material for raising seedlings through tissue culture technology.

Attempts made to develop healthy seedlings using meristem culture technique was not successful. The seedlings raised from meristems excised from BBTV infected plants tested positive in ELISA.

Therefore, the most important step in controlling the disease is to follow strict quarantine regulations even at international level while exchanging of germplasms from one region to another region. Regular and routine indexing of regenerated plants for BBTV incidence through PCR assay or ELISA or ISEM may be made mandatory. Planting of healthy material, selection of explants for tissue culture with full and intensive examination of mother plant is necessary. Rouging of infected plants in field and clean cultivation is advocated. Replacement of infected plants by healthy plants could also reduce the total economical loss.

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*Original not seen.

APPENDIX

APPENDIX - I

Composition of Murashige and Skoog (MS) basal media (Murashige and Skoog, 1962)

Components	Concentration (mg per litre)
Macronutrients	
KNO_3	1900
NH_4NO_3	1650
$CaCl_2.2H_2O$	440
$MgSO_4.7H_2O$	370
KH_2PO_4	170
Micronutrients	
$MnSO_4.4H_2O$	22.3
$ZnSO_4.7H_2O$	8.6
H_3BO_3	6.2
KI	0.83
$CuSO_4.5H_2O$	0.25
$Na_2MoO_42H_2O$	0.25
$CaCl_2.2H_2O$	0.25
$FeSO_4.7H_2O$	27.8
Na_2EDTA	37.3
Vitamins	
Myo-Inositol	100
Thiamine HCL	0.1
Nicotinic acid	0.5
Pyriodoxine HCL	0.5
Glycine	2.0
Sucrose	30000

STUDIES ON BANANA BUNCHY TOP DISEASE

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ABSTRACT

Banana (*Musa* spp.) is one of the most important tropical fruit crops of the world. Among viral diseases of banana, bunchy top is one of the major diseases causing greater economic loss. Survey revealed the presence of disease in all three districts *viz.* Belgaum, Dharwad and Haveri. Incidence was high in Belgaum district followed by Dharwad (0.69%) and Haveri (0.56%) district.

Among different cultivars under cultivation in these districts, G-9 was more susceptible with incidence of 11.11 per cent followed by Rajapuri (4.16%).

The disease was noticed in all most all stages of crop growth symptoms were severe on early infected plants. Colonization of aphids was also noticed in some plantation, which acts as main means for natural transmission of BBTV.

For symptomatalogy, artificially inoculated plants of Robusta cultivar in glasshouse developed symptoms after 6 months. Such plants remained stunted and produce dark-green, narrow, erect and brittle leaves.

The BBTV was transmitted by *Pentalonia nigronervosa* but not by sap inoculation. Infected suckers acts as a main source of primary infection.

A DAS-ELISA technique was standardized using antibodies raised against BBTV and homologous conjugate procured commercially from Suyog Diagnostics Pvt. Ltd., New Delhi. The test detected the virus at 1:10 dilution of sample, 1:100 dilutions of antibody and conjugate. Attempts made to develop healthy seedlings using meristem culture technique was not successful.