PURIFICATION AND IMMUNODETECTION OF BANANA BRACT MOSAIC VIRUS

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DECLARATION

I hereby declare that this thesis entitled "**Purification and immunodetection of banana bract mosaic virus**" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title, of any other university or society.

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Dhanya, M.K.

LIST OF ABBREVIATIONS

µg	Microgram
µl	Micro litre
'A' genome	Acuminata genome
APS	Ammonium persulphate
BA	Benzyl adenine
BBrMD	Banana bract mosaic disease
BBrMV	Banana bract mosaic virus
BBTV	Banana bunchy top virus
'B' genome	Balbisiana genome
BHT	Betylated hydroxy toluene
BRS	Banana research station
BSA	Bovine serum albumin
CVC	Clarified viral concentrate
DAC-ELISA	Direct antigen coating – ELISA
DAS-ELISA	Double antibody sandwich – ELISA
DI	Disease incidence
DIBA	Dot immuno binding assay
DIECA	Sodium diethyl dithiocarbomate
EDTA	Ethylene diamine tetra acetic acid
g	Gram
IAA	Indole acetic acid
IgG	Immuno globulin G
ISEM	Immuno sorbent electron microscopy
L-DOPA	L-3,4-dihydroxy phenyl alanine
M	Molar
MAP	Months after planting
mg	Milligram
min	minute
mM	Milli molar
mm	Millimetre
Mr	Molecular mass
MS media	Murashige Skoog media

LIST OF ABBREVIATIONS CONTINUED

N	Normal
NCM	Nitro cellulose membrane
OD-phenol	Ortho dihydroxy phenol
P	Panchayath
PAL	Phenyl alanine ammonialyase
PDI	Per cent disease index
PEG	Poly ethylene glycol
PO	Peroxidase
PPO	Polyphenol oxidase
PR protein	Pathogenesis related proteins
PSMV	Pigeon pea sterility mosaic virus
PTA-ELISA	Plate trapped antigen – Enzyme linked immunosorbent assay
PVP	Poly vinyl pyrrolidone
Rm	Relative mobility
SDM	Spray dried milk
SDS-PAGE	Sodium dodecyl sulphate – poly acrylamide gel electrophoresis
SPFMV	Sweet potato feathery mottle virus
T	Taluk
TBS	Tris buffer saline
TEM	Transmission electron microscopy
TEMED	Tetramethyl ethylene diamine
TSWV	Tomato spotted wilt virus
W	Weight

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INTRODUCTION

1. INTRODUCTION

Banana and plantains (*Musa* spp.) find place in everyday dishes as desserts and carbohydrate rich food source. The crop is grown extensively throughout the tropical and subtropical region of the world with India third in area (0.464 million hectare) and first in production contributing one third of total fruit production (Shikamany and Sudha, 2004). In Kerala, the area under banana cultivation is 1,06054 ha and the production is 7,69085 tonnes.

Diseases and pests of *Musa* spp. are major constraints to banana production. Among the various diseases, virus diseases of banana are very devastating and considered as potential threat to banana cultivation. Among them, three viruses have been characterized *i.e.*, banana bunchy top virus (BBTV), banana streak virus (BSV) and cucumber mosaic virus (CMV). A fourth virus disease, banana bract mosaic disease (BBrMD) locally known as Kokkan in Kerala, was first observed on Nendran cv. by Samraj *et al.* (1966) at Thrissur district of Kerala. Majority of the commonly cultivated varieties of banana in Kerala are being threatened by this fast spreading menace. The disease was formerly restricted to certain pockets of Thrissur but now it is present in almost all parts of Kerala.

The symptoms of the disease appear first on the leaf sheath as longitudinal, irregular, reddish streaks of varying size. At a later stage, the pseudostem becomes abnormally red in colour and spongy in texture. The affected plants bear leaves of almost normal size but the outer leaf sheaths get detached gradually from the pseudostem. The reddish streaks are formed on the peduncle and bract also. Subsequently the affected plants produce poorly developed bunches. The fingers are very short and undersized having an ashy colour with poor quality. The

characteristic mosaic symptom on the flower bracts gives the disease its common name, banana bract mosaic disease (BBrMD).

BBrMD is found in plants of all age groups and is primarily transmitted through infected suckers and the secondary spread is by the viruliferous aphid *Pentalonia nigronervosa* Coq. The widespread incidence of this disease in Kerala is causing heavy reduction in yield to the tune of about 40 per cent. Yield loss is severe when the infection occurs at the early stages of crop growth. The pathogen involved in the BBrMD is a filamentous virus of the *potyvirus* group (Caruana, 1997).

The BBrMV poses a considerable quarantine risk due to its limited distribution and its ability to spread either through vegetative planting material or by aphids. BBrMV may be more widespread than originally perceived because of confusing symptomatology, complex infection with other banana viruses and the inherent difficulties in detecting viruses in banana sap (Rodoni *et al.*, 1997).

BBrMV has neither been transmitted by any means to hosts outside the genus *Musa* nor to any host by mechanical inoculation making the biological purity of isolates difficult and hampering fulfillment of Koch's postulate (Thomas *et al.*, 1997).

The symptoms caused by BBrMV may be difficult to detect in non-bunching plants in the field. Leaf and petiole symptoms may be inconspicuous and in the absence of the bract the only symptom may be those on the pseudostem covered by the dead outer leaf sheath (Thomas and Magnaye, 1996).

The difficulty to obtain large number of uniform disease free plants with high yield potential by the conventional propagation technique is another major limiting factor in increasing productivity of banana.

In view of the fast spreading nature of BBrMV in Kerala and causing considerable economic loss to the farmers who venture into

banana cultivation procuring loans from commercial banks and also in view of the fact that sufficient informations are at present lacking on etiology, detection and management of the disease, the present study was taken up with the following objectives.

1. Surveying Thiruvananthapuram district to study the prevalence of the disease.
2. Transmission studies to understand spread of BBrMV in field.
3. Pathophysiological studies to understand the mechanisms of damage.
4. Purification and characterization to study the details of BBrMV.
5. Development of immunodetection technique for the early detection of BBrMV.
6. Varietal screening and management of the disease through meristem culture.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Banana cultivation in India is being hampered by a fast spreading menace, banana bract mosaic disease (Kokkan). The disease was first reported on Nendran variety from Kerala by Samraj *et al.* (1966). In 1989, Frison and Putter confirmed the causal agent of the disease as a virus. Magnaye and Espino (1990) found that the shape of banana bract mosaic virus (BBrMV) was filamentous, flexuous rod, belonging to the *potyvirus* group. Banana bract mosaic disease was subsequently observed on many other groups of banana (Reddy *et al.*, 1996).

2.1 SURVEY

Espino *et al.* (1990) conducted a survey and found that banana bract mosaic disease (BBrMD) was wide spread in Philippines. Roperose and Magnaye (1991) estimated yield loss upto 40 per cent due to the disease.

High incidence of banana bract mosaic disease was observed in Kerala and Coimbatore (Thangavelu and Singh, 1996).

Kurien (1998) found that 15 per cent of severely infected banana plants came to shooting whereas 100 per cent shooting occurred in mild and healthy plants. Plants which showed mild symptoms produced bunches with drastic reduction in yield.

During a survey in Maharashtra, banana bract mosaic virus (BBrMV) incidence was found to be low in the tissue culture banana plantation in the farmers' field (Singh and Verma, 2001).

2.1.1 Disease Incidence (DI)

A survey conducted in Trichy by Thangavelu and Singh (1996) revealed that there was 67.13 per cent disease incidence of BBrMD on Nendran varieties.

In another survey carried out in Trichy district to find out the presence of BBrMD in different varieties it was revealed that viral symptoms were observed in cultivars *viz.*, Robusta, Poovan, Ney Poovan, Nendran and the per cent incidence of the disease was 4.07, 3.69, 1.39 and 2.22 respectively. In Malliampathi Nendran orchard, BBrMV incidence was 44 per cent at the early growth stages. After bunch emergence, it increased to 58.10 per cent and at the time of maturing of the bunch, it was 60 per cent (Selvarajan, 1997).

Smitha (2001) conducted a survey in two wards of Kalliyoor panchayaths of Thiruvananthapuram district and found that the two wards were on par in the intensity of the disease and the infection of Red banana and Nendran was found to be significantly higher than Robusta, Palayankodan and Rasakadali.

2.1.2 Per cent Disease Index (PDI)

The PDI on Nendran due to BBrMV infection was 67.13 (Thangavelu and Singh, 1996).

According to Selvarajan (1997) the PDI of BBrMD in Robusta and Nendran was 38.50 and 34.50 respectively.

Sundararaju *et al.* (1999) reported that the PDI of BBrMD on Poovan, Nendran, Neypoovan, Robusta and Rasthali varied from 0.33 to 60.10.

2.1.3 Varietal Reaction

Many local cultivars were found to be affected by BBrMV but was common in Saba and Cardaba clones (Musa BBB). The disease was also common in cavendish varieties (Musa AAA) and there was a serious outbreak in Cotabato in Southern Philippines which resulted in the eradication of about 25,000 plants (Espino *et al.*, 1990).

Thomas and Magnaye (1996) recorded yield losses upto 40 per cent in cv. Cardaba and cv. Lakatan in Philippines. According to them, BBrMV

occurred on a wide range of banana genotypes and was especially wide spread in the varieties Cardaba (ABB/BBB), Saba (BBB) and Abutilon (BB). They could detect the virus in plants of Embul (AAB Mysore) showing the same symptom as in Srilanka.

2.1.4 Symptomatology

Estelitta *et al.* (1987) reported that majority of the BBrMD affected plants exhibited symptoms of varying intensities on pseudostem, leaf sheath, midrib of leaves, spathe, boot leaf, peduncle, male bud and even on fingers. According to them, the symptoms like unusual separation of leaf sheath at the base of plants and travellers' palm configuration of leaves could not be considered as characteristic symptoms of Kokkan disease.

The diagnostic symptom of the disease was the spindle shaped streaks found on the pseudostem after removal of the dried leaf sheaths. As the disease progresses, similar discolourations were also seen on the bracts of the male inflorescence (Castillo and Martinez, 1961). Magnaye and Espino (1990) coined the name banana bract mosaic for the conspicuous discolouration and necrotic streaks that develop on the bracts of male bud.

George *et al.* (1993) identified the pigment in Kokkan affected banana plants as anthocyanin.

The symptoms were more pronounced on young plants and also on suckers of the affected banana. As the disease advances, the streaks turn brownish to black and necrotic. Outer leaf sheaths showed abnormal detachment from the pseudostem. The affected plants produced small bunches with curved pale green to ashy green small fingers. Some of the infected plants showed fan shaped orientation of leaves (Bateson and Dale, 1995).

Balakrishnan *et al.* (1996) reported details regarding the occurrence and symptomatology of BBrMV in Kerala. They reported the association

of the disease with changes in aestivation giving the plants the appearance of travellers' palm.

Cucumber mosaic like symptom other than the typical mosaic pattern on bracts was identified in few plants from India, Vietnam and Western Samoa (Thomas and Magnaye, 1996).

Rodoni *et al.* (1997) described symptoms of BBrMD as discontinuous streaks on the bracts of the banana inflorescence. Infection resulted in mottled discolouration of the pseudostem and irregularly scattered spindle shaped streaks along the petiole. Affected leaves showed severe mosaic on the leaf lamina and mottled pattern on the leaf petioles. They also reported that BBrMV infected banana cultivars from the Coimbatore region showed characteristic mosaic symptom on the bract of banana inflorescence whereas infected plants grown in Thiruchirappally region displayed symptoms similar to those associated with cucumber mosaic incited by *cucumovirus*.

2.2 TRANSMISSION

2.2.1 Transmission through Seed Material

Pushkaran *et al.* (1994) found that the disease caused by BBrMV could only be controlled by the use of healthy planting material. Bateson and Dale (1995) reported the transmission of BBrMV through the infected planting material. According to them the spread of the disease could be checked by the use of disease free suckers. Thomas and Magnaye (1996) observed that the transmission of BBrMV was through vegetative planting material including bits of suckers and tissue cultured plantlets. Estelitta and Radhakrishnan (1996) reported transmission of BBrMV through suckers. Rodoni *et al.* (1997) reported that the spread of BBrMV was through vegetative plant parts. Frison *et al.* (1998) reported the vegetative transmission of BBrMV.

2.2.2 Mechanical Transmission

Rajagopalan (1980) attempted pin-prick and syringe inoculation for the transmission of banana bunchy top virus (BBTV) and found that the result was negative. Muriez (1992) and Dickmann and Putter (1996) found that transmission of BBrMV to hosts outside the genus *Musa* was not possible.

The transmission of BBrMV by mechanical means was unsuccessful and the spread through knives or through handling was unlikely (Thomas and Magnaye, 1996).

According to Thomas *et al.* (1997) no collateral hosts of BBrMV outside *Musa* were known and the virus was not transmissible by mechanical inoculation. No alternative hosts of BBrMV outside *Musa* sp. was known and the virus was not transmitted by mechanical inoculation.

Barbosa *et al.* (1998) reported the successful mechanical transmission of cucumber mosaic virus from banana to indicator plants *viz.*, *Nicotiana glutinosa* and *Cucurbita pepo* cv. *sasertu* producing local lesion and mosaic symptoms in the respective hosts.

2.2.3 Graft Transmission

Sastry *et al.* (1978) reported the successful transmission of BBTV by core grafting under laboratory condition. Rajagopalan (1980) attempted the same but the result was negative.

2.2.4 Insect Transmission

Magnaye and Espino (1990) reported the spread of BBrMD in a non-persistent manner by *Aphis gossypii* and *Rhopalosiphum maidis*.

Bateson and Dale (1995) obtained transmission of BBrMV by the aphid *Pentalonia nigronervosa* Coq. in a non-persistent manner in Philippines. However, transmission studies with the banana aphid *P. nigronervosa* and other insects constantly associated with banana plants

carried out by Sundararaju (1998) gave negative results. Thomas *et al.* (1997) has got successful transmission for BBrMV by banana aphid, *P. nigronervosa*.

Thomas and Magnaye (1996) reported the non-persistent manner of transmission of BBrMV by several aphid species including *A. gossypii*, *P. nigronervosa* and *R. maidis*.

According to Rodoni *et al.* (1997) the spread of BBrMV was through vegetative plant parts and by aphids.

Caruana (1997) reported that the efficiency of transmission of BBrMV by *P. nigronervosa* under laboratory condition was less than 10 per cent.

Selvarajan (2003a) reported 70 per cent transmission of BBrMV by *P. nigronervosa*. According to him BBrMV was non-persistent virus and optimum acquisition feeding period and inoculation feeding period for *P. nigronervosa* was 5 min. and 30 min. respectively.

2.2.5 Soil Transmission

Root samples of Kokkan infected plants were collected and isolation studies attempted could not reveal any fungi or bacteria associated with this disease (Jose *et al.*, 1971). Lockart (1986) found that banana bunchy top disease was not transmitted through soil.

Thomas and Magnaye (1996) reported that the disease caused by BBrMV was not soilborne.

2.3 PATHOPHYSIOLOGY

2.3.1 Carbohydrate

Nair and Wilson (1970) reported higher percentage of carbohydrates in the leaves of banana bunchy top infected plants than the healthy plants.

Fruits of banana plants infected with BBTV contained significantly lower percentage of total carbohydrate, nitrogen, calcium and higher

percentage of sugars and phosphorus than in healthy plants (Jose *et al.*, 1971). Alagiamanavalan *et al.* (1973) reported that there was an increase in reducing and non-reducing sugars and pectin content in BBTV affected fruits.

Rajagopalan (1980) found that carbohydrate content of bunchy top infected banana leaves showed an increasing trend in early stages of infection compared to healthy. But in the later stages it was *vice versa*.

Smitha (2001) reported higher carbohydrate content in the leaves of healthy banana compared to that of BBrMV infected plants. But in bract and flag leaf, carbohydrate content was more in diseased plants.

2.3.2 Chlorophyll

Reduction in total chlorophyll, chlorophyll *a* and chlorophyll *b* due to virus infection was reported by many workers (Johri and Padhi, 1985; Shukla *et al.*, 1992; Sarma *et al.*, 1995; Dantre *et al.*, 1996; Thind *et al.*, 1996).

Smitha (2001) estimated total chlorophyll, chlorophyll *a* and chlorophyll *b* of BBrMV infected banana plants and found that virus infection led to reduction of these items in infected plants compared to healthy.

Yasmin *et al.* (2001) investigated the effect of BBTV on leaf chlorophyll content of banana and found that due to the infection there was severe reduction in total chlorophyll, chlorophyll *a* and chlorophyll *b* over healthy control.

2.3.3 Total Phenol

Rajagopalan (1980) reported reduced accumulation of total phenols in BBTV affected banana plants.

Total phenol was reported to be high in virus infected leaves of many plants (Chakraborthy *et al.*, 1994; Sarma *et al.*, 1995; Dantre *et al.*, 1996; Banerjee and Kalloo, 1998 and Srivastava and Tiwari, 1998).

Smitha (2001) made an observation that the phenol content was significantly influenced by the BBrMD at all stages of growth *i.e.*, the diseased plants had more phenol content compared to healthy ones.

2.3.4 Ortho dihydroxy phenol (OD-phenol)

Rajagopalan (1980) found that OD phenolic content of banana bunchy top infected leaves was higher than the leaves of healthy plant.

2.3.5 Protein

Estelitta (1998) reported that percentage of protein in banana bunchy top affected plant parts such as midrib, leaf lamina, leaf sheath and rhizome was higher compared to that of healthy plants.

Yasmin *et al.* (2001) studied tissue protein of banana cultivar and found that the same in BBTV affected banana plants. According to them the per cent increase over healthy in dwarf Cavendish and William hybrid was 18.90 and 32.1 respectively.

2.3.6 Defence Related Enzymes

Changes in the defence related enzymes due to infection by different viruses have been reported in many plants (Batra and Kuhn, 1975; Wagih and Coutte, 1982; Rathi *et al.*, 1986).

Rajagopalan (1980) observed that banana bunchy top infected plants showed an increasing trend in peroxidase (PO) and catalase activity whereas polyphenol oxidase (PPO) activity was more in healthy plants.

Changes in the terminal oxidases and phenyl alanine ammonialyase (PAL) in banana infected with banana streak mosaic virus (BSV) or banana common mosaic virus were observed by Gomathi *et al.* (1993).

According to them PO, PPO and PAL activity were increased in infected banana plants.

Smitha (2001) found an enhanced activity of PO, PPO and PAL in BBrMV infected banana plants compared to healthy ones.

2.4 ELECTROPHORETIC ANALYSIS OF PROTEINS

Purified BBTV preparations were analysed in 12 per cent Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and visualized by staining with silver nitrate (Wedrychowski *et al.*, 1986).

BBTV proteins were denatured in two per cent SDS and five per cent 2-mercaptoethanol and analysed in discontinuous SDS–polyacrylamide gels. The separated proteins were stained with silver nitrate (Blum *et al.*, 1987).

Wu and Su (1990) determined the relative molecular mass (Mr) of BBTV coat protein subunits by SDS-PAGE (12 per cent) using the system of Laemmli (1970). The molecular mass was estimated by their relative mobilities in the gel.

Thomas *et al.* (1997) determined the relative molecular mass of banana bract mosaic virus coat protein subunits by SDS-PAGE (12 per cent) using the system of Laemmli (1970). Three major protein bands with estimated sizes of 31, 37 and 39 kDa of dissociated BBrMV were obtained.

Estelitta (1998) conducted SDS-PAGE with midribs, petiole, leaf sheath and rhizome portions of healthy as well as BBTV infected banana plants and found that infected tissues contained extra cellular proteins in the midrib portion compared to healthy ones.

2.5 ELECTROPHORETIC ANALYSIS OF ISOZYME

The zymogram pattern of pintobean infected with southern bean mosaic virus exhibited two new PO isozymes in infected plants (Farkas and Stahmann, 1966).

The differences in the isozyme banding pattern were due to variations in the amino acid content of the molecule, which in turn was dependent on the sequence of nucleotides in DNA (Micales *et al.*, 1986).

Among the defence related enzymes PO was the first enzyme to be activated by pathogen attack and other well studied enzyme was esterase (Das *et al.*, 2002).

Sindhu (2001) reported that the isozyme PPO was more prominent in cowpea resistant to black eye cowpea mosaic virus compared to varieties susceptible to the virus.

2.6 BIOASSAY OF ENDOGENOUS GROWTH REGULATORS

Ramasamy (1967) estimated endogenous growth regulator in bunchy top affected banana plants and found that auxin content of infected plant was less compared to healthy. Grieve (1943) came to the same conclusion when working with the tomato spotted wilt virus (TSWV) in tomato. Haibao *et al.* (1997) estimated the level of endogenous hormones of BBTV affected banana plants and found that the gibberlic acid content was lower in diseased than in healthy plants.

2.7 CHARACTERIZATION OF BBrMV

2.7.1 Purification

The method of Rowhani and Stace-Smith (1979) used for purification of potato leaf roll virus was modified and used for the extraction and purification of banana bunchy top virus (BBTV) by Wu and Su (1990). Thomas and Dietzgen (1991) purified BBTV from midrib and petiole tissues modifying the method of Su and Wu (1989).

Espino *et al.* (1990) purified banana bract mosaic virus by density gradient centrifugation in 10-40 per cent sucrose gel as single band. According to him, purified virus had an absorbance ratio of 1.25 at 260/280.

Thomas *et al.* (1997) developed a purification procedure for BBrMV. They purified BBrMV from the frozen leaf midrib and based on the biochemical, serological and cytopathological evidence it was identified as *potyvirus* of 725 nm long, banded at density equivalent to 1.29 – 1.31 g/ ml in cesium chloride equilibrium gradient.

Estelitta (1998) successfully purified BBTV from diseased banana plants as per the procedure of Thomas and Dietzgen (1991).

2.7.2 Electron Microscopy

If the virion is not disfigured or disintegrated upon decoration electron microscopy is valuable in ascertaining the identity of the virus and its serological relationship to others.

Bateson and Dale (1995) through electron microscopic studies found that BBrMV was a filamentous, flexuous rod with a length of 750 nm.

Electron microscopy of BBrMV infected samples showed the presence of large number of flexuous rod shaped virus particles measuring an average length of 750 nm (Singh *et al.*, 1996). According to them, the concentration of the virus was higher in bracts compared to leaf sheath.

Caruana and Galzi (1997) observed filamentous virus particles in BBrMV infected banana by electron microscopy. They reported that partially purified virus preparations of banana contained flexuous rod shaped *potyvirus* like particles measuring approximately 760 x 12 nm.

Rodoni *et al.* (1997) observed flexuous rod shaped *potyvirus* particles measuring approximately 760 x 12 nm using negatively stained, purified BBrMV preparations under a JEOL (Tokyo) 1200E x transmission electron microscope.

Using electron microscopic studies, Thomas and Magnaye (1996) found BBrMV particles in completely symptomless banana plants.

Thomas *et al.* (1997) purified BBrMV and viewed under electron microscope with tobacco mosaic virus as internal size standard and the size of the virions were assumed to be 600 x 18 nm. They processed the tissues for electron microscopy as described by Greber and Gowanlock (1979). Based on biochemical and serological evidence they confirmed that BBrMV was a distinct member of the family *Potyviridae*.

Khiem *et al.* (2000) reported electron microscopy as a technique for studying banana viruses. They observed filamentous thread like particles of BBrMV.

Selvarajan (2003b) developed partially purified BBTV by differential centrifugation and density gradient centrifugation of infected sample. Using this purified virus the antiserum was developed in white hens.

2.7.3 Antiserum Production

Antiserum against BBTV was produced in a New Zealand white rabbit and antiserum titre was determined by gel diffusion (Thomas, 1984).

Thomas *et al.* (1997) developed rabbit polyclonal antiserum against BBrMV using a series of three intramuscular injections of purified virions. Immunoglobulin G (IgG) was prepared from the serum by ammonium sulphate precipitation followed by ion-exchange chromatography and was subsequently used to make alkaline phosphatase conjugate.

Estelitta (1998) successfully produced antiserum against BBTV by giving three intramuscular injections to New Zealand white rabbit with partially purified virus. She detected BBTV in crude extracts of infected banana by indirect ELISA.

Thottappilly *et al.* (1992) purified banana streak virus and successfully developed antiserum against this in mice.

Selvarajan (2003b) successfully developed antiserum against BBTV by immunizing white leghorn chickens with purified virus.

2.7.3.1 Determination of Antibody Titre

Perusal of literature revealed that no such studies were conducted on BBrMV so far. The titre of the antiserum developed by Thottappilly *et al.* (1992) using purified banana streak virus was between 1 : 1,00,000 and 1 : 4,00,000. Sathyamoorthy (2002) reported that an antiserum of titre 1 : 1000 was developed by immunizing white leghorn chicken with purified BBTV.

2.7.4 Immunological Studies

2.7.4.1 Chloroplast Agglutination Test

Estelitta (1998) reported that chloroplast agglutination test was found to be successful for virus detection from crude sap of banana infected with BBTV and the precipitate was visible in transmitted light.

2.7.4.2 Agar Gel Double Diffusion Test

Moyer and Kennedy (1978) performed double diffusion tests and found precipitin bands at an antiserum dilution of 1:16 with sweet potato feathery mottle virus (SPFMV) in infected extracts of sweet potato and no precipitin lines in healthy extracts.

In double diffusion tests, the virus gave sharp continuous precipitin bands against potato virus Y antisera (Khurana *et al.*, 1987).

Estelitta (1998) found that agar gel diffusion test of crude sap of BBTV infected banana was not successful whereas with purified virus preparations, clear precipitin bands were developed.

2.7.4.3 Microprecipitin Tests on Slides

Perusal of literature showed that no such studies were conducted on virus diseases of banana so far. Eloja and Tinsley(1963) successfully conducted microprecipitin test to detect Abaca mosaic virus (AbaMV) in Abaca (Musa textiles) using antiserum developed against AbaMV.

2.7.4.4 Enzyme Linked Immuno Sorbent Assay (ELISA)

Plate trapped antigen - enzyme linked immuno sorbent assay (PTA-ELISA) was used to detect the purified virus antigen (Wu and Su, 1990).

Mariappan and Mathikumar (1992) found direct antigen coating (DAC) ELISA as an efficient method in detecting BBTV even at a very high dilution of 1:1000.

Zhang *et al.* (1995) conducted an indirect ELISA for the detection of banana bunchy top disease with monoclonal antibody of BBTV.

Based on serological tests Reddy *et al.* (1996) revealed that all *potyvirus* antisera could detect BBrMV infection and it was more serologically related to potato virus Y.

Singh *et al.* (1996) detected BBrMV by Double antibody sandwich (DAS) ELISA using antiserum obtained from Philippines. Positive reaction for all symptomatic samples was observed in ELISA.

Thomas *et al.* (1997) developed an ELISA technique to detect BBTV in banana sap diluted to 1:64,000 (equivalent to 16 µg of leaf sample). They confirmed BBrMV as a member of *potyvirus* group by serological studies including ELISA. DAS-ELISA was developed to investigate the BBrMV in infected banana plants. Antibody titre was assessed by PTAC-ELISA and defined as the maximum dilution of antiserum giving a positive reaction with antigen and has an A_{410} value > 0.1 after 90 minutes of substrate incubation. According to them A_{410} value for BBrMV infected sample was 1.16.

Thomas and Magnaye (1996) could detect BBrMV in completely symptomless plants using serological tests *i.e.* ELISA, agar gel double diffusion test.

Geering *et al.* (1997) reported that in Plate trapped antigen coating (PTAC)-ELISA weak serological relationship were demonstrated between BBrMV and other members of family *potyviridae* including Abaca mosaic virus (AbaMV), Dashbean mosaic and Wheat streak mosaic virus.

Ndovora and Lockhart (1997) developed a serological assay for detecting banana streak virus isolate by DAS-ELISA and immuno sorbent electron microscopy (ISEM).

2.7.4.5 Dot Immuno Binding Assay (DIBA)

Selvarajan (1997) successfully used dot immuno binding assay (DIBA) for the detection of BBrMV in infected banana plants. Definite blue coloured dots were developed for infected samples on nitrocellulose membrane whereas it was absent in healthy samples.

2.8 MANAGEMENT OF THE DISEASE

2.8.1 Screening for Resistance

Estelitta *et al.* (1992) screened 152 varieties against banana bract mosaic virus. They found that 34 varieties showed the characteristic symptoms of BBrMV infection. According to them, the varieties having 'B' genome were found to be more susceptible to the disease. The pure acuminata varieties such as Pisanglin (AA), Manoranjitham (AAA), Sanna Chenkadali (AA) and Robusta (AAA) were not susceptible.

The screening of varieties against the disease indicated that the varieties having 'B' genome were more susceptible to BBrMV compared to those with 'A' genome (pure acuminata varieties) (Estelitta and Radhakrishnan, 1996).

Thangavelu and Singh (1996) reported BBrMV on Monthan group of banana in Coimbatore and Kovur. Survey conducted by them in Trichy revealed the co-existence of BBrMV and BSV in the cultivar Poovan. According to them incidence of BBrMV varied from 0.2 to 8.8 per cent in Nendran. The disease incidence was also noted in Robusta and Ney Poovan while it was negligible in Monthan cultivars.

Caruana (1997) was of the opinion that control of vectors and the use of virus free planting materials were the ways of controlling the disease.

Frison *et al.* (1998) recommended the use of healthy planting material for the management of BBrMD.

Cherian *et al.* (2002) conducted studies on yield reduction due to BBrMV infection on Nendran (AAB), Mysore (AAB), Robusta (AAA), BRS-1 (AAB) and Neypoovan (AB) and reported that the yield reduction varied with varieties. Maximum variation was in Robusta (70 per cent) followed by Nendran 52.5 per cent. Besides yield reduction fingers became malformed.

2.8.2 Virus Elimination through Meristem Culture

Thomas *et al.* (1997) reported that symptomatology of various virus diseases of banana was very unreliable for screening micropropagated plants whereas ELISA was reliable. According to them tissue culture could be used to eliminate BBTV from the germplasm. Singh and Verma (2001) indexed BBrMV incidence in tissue culture banana plantation in farmers field using polyclonal antiserum from Agdia, Incorporated, Elkhart, Indiana 46514 USA and recorded low incidence of BBrMV *i.e.*, 4.1 per cent.

Shinchaun and Hongii (1998) found that banana plantlets produced by tissue culture in Taiwan were free of virus and a total of 26 million plants were produced under this programme.

According to Frison *et al.* (1998) viral diseases of banana could be eliminated by meristem tip culture in combination with thermotherapy.

Rames and Zamore (1999) recommended heat treatment of BBrMV infected shoot culture of banana for 2 – 8 weeks prior to regeneration of plants. Isolated meristems of these treated plants yielded high per cent of BBrMV free plants. According to them heat treatment of micropropagated plants for one week coupled with serological indexing was the fastest route to obtain BBrMV free plants.

According to Sathyamoorthy (2002) meristem culture technique using meristems of plants positive to BBrMV in DIBA is in progress in Trichy.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

The present study on banana bract mosaic virus (BBrMV) causing the disease popularly known as Kokkan in banana was carried out during 2000-2003 period. Experiments were conducted at the College of Agriculture, Vellayani using Nendran variety and the survey was conducted in the farmers' fields of Thiruvananthapuram district.

3.1 SURVEY

A detailed survey was conducted to study the incidence of banana bract mosaic virus disease (Kokkan) in the banana growing areas of Thiruvananthapuram district following stratified two stage random sampling technique. The strata were taken as four taluks *viz.*, Nedumangadu (T_1), Neyyattinkara (T_2), Thiruvananthapuram (T_3) and Chirayinkeezhu (T_4) of the Thiruvananthapuram district. From each taluk three panchayaths were selected and from each panchayath five plots having a minimum of 100 plants were selected. A total number of 6,000 plants were observed. The data on disease incidence and per cent disease index were collected twice from each plot at six months interval.

3.1.1 Disease Incidence (DI)

The per cent of disease incidence was calculated by recording the number of plants infected out of 100 plants in each plot.

3.1.2 Per cent Disease Index (PDI)

Per cent disease index of each plot was calculated as per Mayee and Datar (1986) using 0-4 scale as described below.

0 – No symptoms

1 – Reddish brown streaks on 1-25 per cent area of the pseudostem

2 – Reddish brown streaks on 26-50 per cent area of the pseudostem

3 – Reddish brown streaks on 51-75 per cent area of the pseudostem

4 – Reddish brown streaks on more than 75 per cent area of the pseudostem

$$PDI = \frac{\text{Sum of individual grades}}{\text{Number of plants assessed}} \times \frac{100}{\text{Maximum grade}}$$

3.1.3 Varietal Reaction

Observation was made to study the susceptibility of different banana varieties to BBrMV based on the visual symptoms.

3.1.4 Symptomatology

During the survey different types of symptoms developed in different varieties were recorded.

3.2 TRANSMISSION

All the transmission studies of BBrMV were carried out under glasshouse condition.

3.2.1 Transmission through Seed Material

Suckers were collected from both infected and healthy plants (10 each) and were planted in pots for three seasons. These plants were sprayed with 0.05 per cent monocrotophos and kept in insect proof glasshouse for three months and expression of symptoms were observed.

3.2.2 Mechanical Transmission (Sap Transmission)

For preparing the inoculum one part of BBrMV infected leaf tissue was homogenized with one part of pre-cooled buffer using a chilled mortar and pestle. The following buffers were used for transmission studies (Table 1 and Appendix I).

Table 1 Different buffers used for sap transmission

Buffer (0.1 M)	pH
Sodium borate buffer	8.0
Potassium phosphate buffer with and without sodium diethyl dithiocarbomate	7.2
Sodium phosphate buffer	7.2
Tris buffer	7.2
Citrate buffer	7.2

The homogenate was filtered through a thin layer of absorbent cotton to clarify the inoculum and maintained in chilled condition. Mechanical inoculation was performed using the following methods.

3.2.2.1 Sap Inoculation by Leaf Rubbing

The inoculum was rubbed on the young emerged leaves of ten healthy Nendran plants grown in pots. Prior to inoculation, the leaves of test plants were uniformly dusted with carborundum powder (600 mesh) and inoculated by gently rubbing on the upper surface of the leaves using the forefinger moistened with the inoculum. Any excess inoculum was removed by washing with water and the inoculated plants were kept in an insect proof glasshouse. Plants were observed for the development of symptoms upto three months.

As described above sap transmission was attempted to transmit BBrMV to local lesion hosts like *Nicotiana tabacum* and *Nicotiana benthamiana*.

3.2.2.2 Sap Injection Using Syringe

The virus inoculum was taken in a syringe and was injected into the pseudostem of ten healthy plants. Injection of healthy plants with leaf extract prepared from healthy plant served as control.

3.2.2.3 Pin-prick Inoculation

Prior to inoculation with BBrMV inoculum, pin-pricks were made on spindle leaf of ten healthy Nendran plants using sterilized entomological needles and on these leaves, inoculum was rubbed using forefinger moistened with the inoculum. These suckers were maintained in an insect proof glasshouse for three months for symptom expression.

3.2.2.4 Inoculation Using Cutting Knives

Using a knife the young leaves of ten BBrMV infected Nendran plants were removed. The same knife was used to remove the emerging leaves of ten healthy Nendran suckers. Detopped healthy Nendran suckers were maintained in an insect proof glasshouse for three months for symptom expression.

3.2.3 Graft Transmission

3.2.3.1 Core Grafting

Using a cork borer of 2.5 cm diameter cores were taken from rhizomes of ten BBrMV infected suckers. Using the same cork borer rhizomes of ten healthy Nendran suckers were pierced and the cores were removed wherein cores of infected rhizome were inserted. After insertion the wound portion was plastered with a paste of bavistin. These grafted suckers were freshly planted in pots, maintained in an insect proof glasshouse for three months and observed for symptom development.

This type of grafting was also attempted in already established healthy Nendran suckers in pots by removing the soil and by exposing the rhizome.

3.2.3.2 Root Grafting

Pots of both healthy and infected Nendran suckers (10 each) were placed side by side. The top soil of the pot was carefully removed and the roots were exposed and a slanting cut was made in the root at about one

centimetre away from the root tip of infected sucker. A similar cut was made on the roots of the healthy sucker also. The cut portions of both healthy and infected roots were tied together immediately using a polyethylene strip. Grafted suckers were maintained for three months in an insect proof glasshouse and observed for symptom expression.

3.2.4 Insect Transmission

3.2.4.1 Collection and Rearing of Vector

The insect vectors *viz.*, *Pentalonia nigronervosa* Coq., *Aphis gossypii* and *Aphis craccivora* were used for transmission studies.

The aphid, *P. nigronervosa* was reared on small healthy banana suckers planted in pots and kept under shade. Aphids on the healthy banana plants were gently tapped and disturbed and then collected with the help of a camel hair brush into a petri dish. After the collection the mouth of the petri dish was covered with muslin cloth. These insects were then released singly into the leaf axils of healthy banana suckers using the camel hair brush for multiplication. Suckers each infested with a single aphid were maintained in separate cages in glass house. Non-viruliferous aphids thus reared in the insect proof glass house were used for transmission studies.

BBrMV infected Nendran plants were collected from the Instructional Farm, Vellayani and were planted in pots. The virus culture was maintained on banana in an insect proof glass house by periodical inoculation of healthy suckers using viruliferous insect vector *P. nigronervosa* (Banana aphid).

Healthy colonies of *A. gossypii* were established on brinjal and was maintained in glasshouse for transmission studies. BBrMV infected Nendran plants served as the source for acquisition of the virus. Healthy colonies of *A. craccivora* were maintained on cowpea plants.

3.2.4.2 Transmission through Aphid Vectors

Healthy aphid (*P. nigronervosa*) colonies maintained on disease free Nendran suckers were used for transmission. Using a camel hair brush they were collected in a petri dish. The mouth of the dish was covered using muslin cloth and allowed to starve for one hour. The starved aphids were allowed to feed on detached BBrMV infected Nendran leaves and pseudostem for 30 minutes for acquisition feeding. The viruliferous aphids were transferred to one month old healthy banana plants @ 20 per plant. After inoculation feeding period of 24 hour the insects were killed by spraying monocrotophos (0.05 per cent).

In the case of *A. gossypii* and *A. craccivora* pre-acquisition fasting for one hour and acquisition feeding period of 10 minutes and inoculation feeding period of 24 hour were given. Twenty viruliferous aphids per plant were used for each study.

3.2.4.2.1 Effect of pre-acquisition Fasting of *P. nigronervosa* on the Efficiency of Transmission of BBrMV

Groups of 20 non-viruliferous *P. nigronervosa* (both nymphs and adults) were fasted for various intervals of time *viz.*, 5, 15, 30 minutes, one hour and two hours. A group of 20 unstarved aphids served as control. Then the vectors were allowed to feed on diseased plant parts for 30 minutes to acquire the virus. After the acquisition feeding the vectors were transferred to young healthy banana plants for 24 hour for inoculation feeding. At the end of inoculation feeding period the insects were killed by spraying monocrotophos (0.05 per cent).

3.2.4.2.2 Acquisition Threshold of the Vector *P. nigronervosa*

To determine the minimum acquisition feeding period required to render the aphids viruliferous, groups of 20 insects were starved for one hour and allowed to feed on diseased plant parts for different periods *viz.*, one min, 15 min, 30 min, 45 min and one hour. At the end of acquisition

feeding period the aphids were transferred to one month old healthy banana plants and allowed to feed for 24 hour for inoculation feeding. Then the insects were killed and plants were kept under observation in insect proof glasshouse.

3.2.5 Soil Transmission

Healthy Nendran suckers were planted in pots containing the soil collected from the basins of BBrMV infected plants. These plants were kept in glasshouse under insect proof condition for three months. Healthy suckers planted in pots containing the soil collected from disease free areas served as control.

3.3 PATHOPHYSIOLOGY

Physiological changes in banana as a result of BBrMV infection were studied. The data were statistically analysed to find out whether there was any significant physiological changes in BBrMV infected plants.The leaf samples were collected from both the infected and healthy Nendran banana plants and total carbohydrate, chlorophyll, total phenol, OD-phenol and protein were estimated. The activities of defence related enzymes at different growth stages *i.e.*, three months after planting (3 MAP), six months after planting (6 MAP), flag leaf stage and bract (flowering stage) were analysed and bioassay of endogenous growth substances at fruiting stage were also conducted.

3.3.1 Total Carbohydrate

Carbohydrate content was estimated following the method of Hodge and Hofreiter (1962).

One hundred milligrams of leaf sample was weighed into a boiling tube and hydrolysed with five ml of 2.5 N HCl by keeping it at 100°C in a water bath and then cooled to room temperature. The hydrolysate was neutralized with solid sodium carbonate (Na_2CO_3) until the effervescence ceased. Later the volume was made upto 10 ml with distilled water and

centrifuged at 10,000 rpm for 10 minutes. To 0.5 ml of the supernatant, 0.5 ml of distilled water and four ml of anthrone reagent (200 mg anthrone in 100 ml of ice cold 95 per cent H_2SO_4) were added. The reaction mixture was heated for eight minutes at 100°C in water bath and cooled rapidly. When the colour of the solution became green to dark green, the absorbance was determined at 630 nm using a Systronics UV-VIS spectrophotometer model - 118. Amount of carbohydrate present was calculated from standard curve prepared using D-glucose and expressed in terms of milligrams of glucose equivalent per gram of leaf tissue on fresh weight basis.

3.3.2 Chlorophyll

Chlorophyll was estimated by the method described by Arnon (1949). One gram of leaf sample (W) was finely cut and ground in a mortar with 20 ml of 80 per cent acetone. The homogenate was centrifuged at 5000 rpm for five minutes and the supernatant was transferred to a 100 ml volumetric flask. The above procedure was continued till the residue became colourless. The final volume (V) in volumetric flask was made upto 100 ml. Absorbance of the solution at 645 (A 645) and 663 (A 663) nm was determined using a spectorphotometer (Systronics UV-VIS Spectrophotometer-118) against the solvent (80 per cent acetone) as blank. The chlorophyll content was calculated using the following equations and expressed as milligrams of chlorophyll per gram of leaf tissue.

$$\text{Total chlorophyll} = 20.2\ (A645) + 8.02\ (A663) \times V / 1000 \times W$$

$$\text{Chlorophyll a} = 12.7\ (A663) - 2.69\ (A645) \times V / 1000 \times W$$

$$\text{Chlorophyll b} = 22.9\ (A645) - 4.68\ (A663) \times V/1000 \times W$$

3.3.3 Total Phenols

Phenol content was estimated following the procedure of Bray and Thorpe (1954). One gram leaf sample was ground in 10 ml of 80% ethanol

using a pestle and mortar. The homogenate was centrifuged (Hettich, EBA 12/12I) at 10000 rpm for 20 minutes. The supernatant was saved and the residue was re-extracted with five times the volume of 80% ethanol and re-centrifuged. The supernatant was collected and pooled together before evaporating to dryness. The resulting residue was dissolved in five ml of distilled water. An aliquot of 0.1 ml of this sample was pipetted out into a test tube and the volume was made upto three ml with distilled water. Folin-Ciocalteau reagent (0.5 ml) and two ml of 20 per cent sodium carbonate solution were added to each tube after three minutes. The reaction mixture was thoroughly mixed and kept in boiling water for one minute. After cooling the absorbance was measured at 650 nm against a reagent blank using a spectrophotometer (Systronics UV-VIS spectrophotometer model - 118). A standard curve was prepared and phenol content was expressed as catechol equivalent per gram fresh weight of leaf.

3.3.4 Ortho-Dihydroxy Phenol (OD-phenol)

OD-phenol was estimated as per the procedure described by Johnson and Schaal (1957).

Three grams each of healthy and infected leaf sample were boiled for five minutes in 12 ml of 80 per cent ethanol. After cooling, the tissue was ground using a pestle and mortar and the homogenate was centrifuged (Hettich, EBA 12/12 R) at 10,000 rpm for 20 minutes. The supernatant was saved and the residue was re-extracted with 80 per cent ethanol and centrifuged again. The supernatant was collected, pooled and the final volume was adjusted to 15 ml with 80 per cent ethanol. One ml of 0.5 N HCl, one ml of Arnons reagent and two ml of 1 N NaOH were added to one ml of the extract and the volume was made upto 25 ml with 80 per cent ethanol (Appendix II). The absorbance was measured at 540 nm in a UV-VIS spectrophotometer (Systronics UV-VIS spectrophotometer model-118). A standard curve was prepared by using different

concentrations of catechol (30, 40, 50, 60, 70 and 80 µg) and OD-phenol content was expressed as catechol equivalent per gram fresh weight of leaf.

3.3.5 Protein

Total soluble protein content was estimated as per the procedure described by Bradford (1976).

One gram of leaf sample was homogenized in three ml of 0.1 M sodium phosphate buffer (pH 7.0) using a pre-chilled pestle and mortar. The extract was centrifuged at 10,000 rpm for 10 minutes at 4°C and the supernatant was used for the experiment.

Water and working dye solution (0.5 and 5 ml respectively) was added to 0.5 ml of the extract and incubated for five minutes at room temperature. One ml of water with five ml of dye solution served as control. Reading was taken at 595 nm in a spectrophotometer. Bovine serum albumin (BSA) was used as the protein standard. The protein content was expressed as microgram albumin in equivalent of soluble protein per gram on fresh weight basis (Appendix III).

3.3.6 Defense Related Enzymes

3.3.6.1 Peroxidase (PO) Activity

Peroxidase activity was determined according to the procedure described by Srivastava (1989). Leaf sample of 200 mg was homogenized in one ml of 0.1 M sodium phosphate buffer (pH 6.5) to which a pinch of polyvinyl pyrrolidone (PVP) was added. The homogenization was done at 4^0C using a mortar and pestle. The homogenate was filtered through a muslin cloth and centrifuged at 5000 rpm for 15 minutes at 4^0C. The supernatant was used as the enzyme extract for the assay of PO activity (Appendix IV).

The reaction mixture consisting of 1 ml 0.05 M pyrogallol and 50 µl of enzyme extract was taken in both reference and sample cuvettes, and kept in a spectrophotometer (Systronics UV-VIS spectrophotometer 118) and the reading was adjusted to zero at 420 nm. The enzyme reaction was started by adding one ml of one per cent hydrogen peroxide (H_2O_2) into sample cuvette and change in absorbance was measured at 30 seconds interval.

3.3.6.2 Polyphenol Oxidase (PPO) Activity

Polyphenol oxidase activity was determined as per the procedure given by Mayer *et al.* (1965). The enzyme extract was prepared as per the procedure given for the estimation of peroxidase.

The reaction mixture contained one ml of 0.1 M sodium phosphate buffer (pH 6.5) and 50 µl of enzyme extract. The reaction was initiated after adding one ml of 0.01 M catechol. The observations were recorded in a spectrophotometer (Systronics UV-VIS spectrophotometer model - 118). The change in absorbance was recorded at 495nm and PPO activity was expressed as change in the absorbance of the reaction mixture per minute per gram on fresh weight basis (Appendix IV).

3.3.6.3 Phenylalanine Ammonialyase (PAL) Activity

PAL activity was analysed based on the procedure described by Dickerson *et al.* (1984). The enzyme extract was prepared by homogenizing one gram leaf sample in five ml of 0.1 M sodium borate buffer (pH 8.8) containing a pinch of PVP using chilled mortar and pestle. The homogenate was centrifuged at 10000 rpm for 10 minutes at 4^0C. The supernatant was used for the assay of PAL activity. The reaction mixture contained three ml of 0.1 M sodium borate buffer (pH 8.8), 0.2 ml of enzyme extract and 0.1 ml of 12 mM L-phenyl alanine prepared in the same buffer. The blank contained three ml of 0.1 M sodium borate buffer (pH 8.8) and 0.2 ml enzyme extract. The reaction mixture and blank was incubated at 40^0C

for 30 minutes and reaction was stopped by adding 0.2 ml of 3N hydrochloric acid. The absorbance was read at 290 nm in a spectrophotometer (Systronics UV-VIS spectrophotometer 118) (Appendix V).

PAL activity was expressed as nanomoles of cinnamic acid produced per minute per gram on fresh weight basis.

3.4 ELECTROPHORETIC ANALYSIS OF PROTEINS

3.4.1 Characterization of Proteins by Sodium Dodecyl Sulphate Poly Acrylamide Gel Electrophoresis (SDS-PAGE)

Electrophoretic separation of soluble protein of banana leaves were carried out as per procedure described by Laemmlli (1970). Leaf samples of healthy and BBrMD affected plants were taken for analysis.

Five hundred milligram each of healthy and infected leaf samples were homogenized in 200 µl of cold denaturing solution at 4°C (Appendix VI). The supernatent was mixed with chilled acetone in the ratio of 1:4 and the protein was allowed to precipitate by keeping the mixture at 4°C for 30 minutes. The sample was centrifuged at 5000 rpm for 15 minutes at 4°C. The precipitate was resuspended in 20 µl of denaturing solution and vortexed. The homogenate was centrifuged at 5000 rpm for 15 minutes. The supernatant was mixed with equal volume of sample buffer and kept in a boiling water bath for three minutes to ensure complete reaction between protein and SDS. These samples were used for SDS-PAGE. The protein concentration was adjusted in each sample to a strength of 100 µg of protein following Bradford method. The following reagents were used for the characterisation of proteins by SDS-PAGE.

a) Acrylamide stock (30 %)

Acrylamide	– 29.2 g
Bis-acrylamide	– 0.8 g
Double distilled water	– 100.0 ml

b) Separating (resolving) gel buffer stock (1.5 M Tris-HCl pH 8.8)

Tris base (18.15 g) was dissolved in approximately 50 ml of double distilled water. The pH was adjusted to 8.8 with 6 N HCl and made up the volume to 100 ml with double distilled water and stored at 4°C.

c) Stacking gel buffer stock (0.5 M Tris-HCl pH 6.8)

Tris base (6.0 g) was dissolved in approximately 60 ml of double distilled water and adjusted the pH to 6.8 with 6 N HCl and the volume was made upto 100 ml with double distilled water and stored at 4°C.

d) Polymerising agents

Ammonium per sulphate (APS) 10 per cent prepared freshly before use.

Tetra methyl ethylene di-amine (TEMED) – Fresh from the refrigerator.

e) Electrode buffer pH 8.3

Tris base	6.0 g
Glycine	28.8 g
SDS	2.0 g
Double distilled water	2 litre

f) Sample buffer (SDS-reducing buffer)

Double distilled water	2.6 ml
0.5 M Tris HCl pH 6.8	1.0 ml
2-mecaptoethanol	0.8 ml
Glycerol	1.6 ml
SDS 20 % (w/v)	1.6 ml
0.5 % Bromophenol blue	0.4 ml

g) Staining solution

Comassie brilliant blue R 250	0.1 g
Methanol	40.0 ml
Glacial acetic acid	10.0 ml
Double distilled water	50.0 ml

h) Destaining solution

As above without Coomassie brilliant blue R 250

Procedure

Separating gel was first casted followed by stacking gel by mixing the various solutions as indicated below.

a) Preparation of separating gel (12%)

Double distilled water	6.7 ml
Tris-HCl, pH 8.8	5.0 ml
SDS 10 %	0.2 ml
Acrylamide stock	8.0 ml

The above solution was mixed well and degassed for three minutes and then the following were added immediately.

10 per cent APS (freshly prepared)	0.10 ml
TEMED	0.01 ml

The separating gel was mixed well and poured immediately between glass plates and a layer of water was added above the polymerizing solution to quicken the polymerization process.

b) Preparation of stacking gel

Double distilled water	6.1 ml
Tris HCl, pH 6.8	2.5 ml
SDS 10 %	0.1 ml
Acrylamide stock	1.3 ml

The solution was mixed well, degassed and the following were added.

APS 10 %	0.05 ml
TEMED	0.1 ml

The water layered over the separating gel was removed and washed with a little electrode buffer and then the stacking gel was poured over the polymerized separating gel, after keeping the comb in position.

After polymerization the samples were loaded into the wells. The electrophoresis was performed at 100 V till the dye reached the separating gel. Then the voltage was increased to 200 V and continued till the dye reached the bottom of the gel. The gel was removed immediately after electrophoresis between the glass plates and incubated in the staining solution for overnight with uniform shaking. Then the gel was transferred to the destaining solution. The protein appeared as bands and the gel was photographed after placing it on a transilluminator (Appligene Model White / UV TMW-20) (Appendix VII).

3.5 ELECTROPHORETIC ANALYSIS OF ISOZYME

Electrophoresis of protein extracts from plant tissues using different kinds of support media and buffer systems allows separation of the multiple forms of enzymes (isozymes) on the basis of charge and molecular size.

Soluble and ionically bound enzymes were extracted by grinding the sample under chilled condition in 50 mM Tris-Cl (pH 7.6) in the ratio of 1:2 w/v. The homogenate was centrifuged at 15,000 rpm for 10 minutes at 4°C. The resulting supernatant was used for isozyme analysis. Total protein content of the sample was determined by Bradford method (Bradford, 1976).

Discontinuous anionic polyacrylamide gel electrophoresis was conducted under non-dissociating conditions as previously described by Wagih and Coutts (1982) with slight modification. Proteins extracted by 50 mM Tris (pH 7.6) were separated by gel electrophoresis in 7.5 per cent gel. The gel was prepared using the stock solutions prepared for isozyme analysis (native gel) (Appendix VIII).

Reagents

a) Separating gel (7.5 %)

Tris chloride buffer stock solution (pH 8.9)	5 ml
Resolving gel acrylamide solution	10 ml
Distilled water	25 ml
APS	300 µl

b) Stacking gel (4%)

Tris chloride buffer stock, pH 6.7	2.5 ml
Resolving gel acrylamide solution	3.1 ml
Distilled water	14.1 ml
APS	300 µl

Following electrophoresis, the gel was immersed in a solution of 10 mM L-3,4 dihydroxy phenylalanine (L-DOPA) in 100 mM sodium phosphate (pH 7.0) in a plastic tray kept in a shaker for 30 minutes. Zones of enzyme activity (PPO) were observed as greyish black bands.

The Rm value and relative intensities of the isozyme bands of PPO were also recorded.

For PO enzyme, gel was incubated in 0.6 M sodium acetate buffer (pH 5.4) containing 0.5 per cent O – Dianisidine HCl for 30 minutes at room temperature. The gel was transferred to 0.1 M hydrogen peroxide until visible bands were developed. The Rm values of PO was also recorded.

3.6 BIOASSAY OF ENDOGENOUS GROWTH REGULATORS

The level of endogenous growth regulators in BBrMV infected fruits were estimated as per the method of Richard (1997).

Fruit extracts of healthy and infected Nendran plants were prepared using a pestle and mortar under cold condition. While grinding a pinch of Betylated hydroxy toluene (BHT) was added to prevent the oxidation of growth regulators. Ground tissue was transferred to five volume of chilled distilled ethanol (80 per cent) and extracted for 24 hours at 4°C. Homogenate was centrifuged at 2500 rpm for five minutes. Residue was re-extracted twice with five volume of ethanol. Filtrate was allowed to evaporate in a rotary evaporator at room temperature. Finally the residue was dissolved in distilled water and was used as the sample for the estimation of different growth regulators.

3.6.1 Estimation of auxin

Wheat coleoptile straight growth bioassay was used for the estimation of auxin.

For this wheat seeds were germinated on a moist filter paper. After germination seeds were transferred to a sand tray and allowed the seedlings to grow at a temperature of 24 - 26°C under high humid conditions for a period of 98 hours in dark. Coleptile after attaining a length of 25 mm were de-toped in darkness and kept as such for five hours to allow the auxin to diffuse down.

Five coleoptile segments of 0.5 cm length were transferred to 10 ml of the sample extracted for growth regulator estimation. For preparing standard curve, coleoptile segments (0.5 cm length) were placed in each of different concentration of auxin ranging from 10^{-4} to 10^{-9} M. Five coleoptile segments on phosphate buffer (pH 5.9) used for preparing the auxin solution served as control. The length of these segments was measured after 24 h. The per cent increase in length over control was measured and values for standards were plotted against the respective auxin concentration and the standard curve was prepared. From this standard curve the levels of IAA in test solution was estimated (Appendix IX).

3.6.2 Estimation of Cytokinin

For the estimation of cytokinin, cucumber cotyledon expansion bioassay was performed. Cucumber seeds were germinated in complete darkness for 48 hours at 28°C. Cotyledons were separated from seed coat and embryonic plumule and radicle were transferred to sterile petriplates containing 10 ml of the test sample at the rate of 12 cotyledons/plate.

Standard curve was prepared by making different concentrations of cytokinin (Benzyl adenine) ranging from 10^{-5} to 10^{-8} M in 0.2 mM Phosphate buffer (pH 5.9) and to 10 ml of each dilution 12 cotyledons were transferred (Appendix X).

Treatments of both sample and standards were kept under a weak fluorescent light (250 lux for three days). Then cotyledons were carefully blotted to dryness and their fresh weights were recorded and the per cent increase in fresh weight over control was calculated. The amount of cytokinin in samples were estimated from the standard graph.

3.6.3 Estimation of Gibberlic Acid

Amount of Gibberlic acid (GA) in the sample was estimated by the method of Richard(1997).

The barley seeds were placed on a moist filter paper for 15 hours. Then they were cut laterally to two halves and the portions with embryo were discarded. The other portions were soaked in both sample and GA solutions of 10^{-4} M to 10^{-10} M in acetate buffer (for standard). De-embryonated half of seeds were incubated for 24 hour. After incubation, seeds were ground in a pestle and mortar with acetate buffer (pH 5.6). Extracted samples were centrifuged at 5000 g for 10 minutes. The clear supernatant was taken as the crude enzyme extract. One ml of seed extract was added to One ml of one per cent starch solution. The mixture was incubated at 25°C to allow starch hydrolysis. The amount of reducing sugar in the mixture was estimated by Dinitro salicylic acid (DNS reagent) method. For this one ml of the above sample was pipetted out into the test tube and was diluted to three ml with distilled water. To each tube three ml of Dinitro salicylic acid reagent was added (Appendix XI). The contents were heated in a boiling water bath for five minutes. One ml of 40 per cent Rochelle salt solution (potassium sodium tartarate) was added to these tubes before cooling. Each sample was cooled and the intensity of the dark red colour was read at 510 nm. A series of standards were also run using glucose (0 to 500 kg) and a graph was plotted. From this graph, extent of starch hydrolysis for different levels of GA and that of sample was estimated from which the amount of GA in the sample was estimated.

3.7 CHARACTERIZATION OF BBrMV

3.7.1 Purification

Purification of BBrMV was carried out using the method described by Khurana *et al.* (1987) for the purification of poty virus with slight modification. The stock culture of BBrMV was maintained in insect proof glass house in pots as infected Nendran suckers. Emerging leaves of the infected suckers were used for purification. Midribs of the leaves were removed and 100 g of infected leaves were put in a poly bag and kept at -

-80°C for one hour. The frozen leaves were ground in a blender under chilled condition using 0.1 M phosphate buffer (pH 7.2) containing 15 mM each of sodium diethyl dithiocarbamate (DIECA) and disodium ethylene diamine tetra acetate (EDTA) in the ratio 1:1.5. The homogenate was strained through double layered muslin cloth and the filtrate was clarified by centrifugation at 5000 rpm for 15 minutes in a refrigerated centrifuge (Hettich EBA 12R). The supernatant was collected and was mixed with equal quantity of chloroform N-butanol mixture (1:1 v/v). It was kept in a shaker at 4°C for half an hour. Then it was re-centrifuged at 5000 rpm for 15 minutes at 4°C. The aqueous phase was collected. To this four per cent polyethylene glycol (PEG MW 6000) and 0.2 M sodium chloride were added and the mixture was kept at 4°C for one hour with constant stirring. This was kept overnight at refrigerated condition. Next day the virus precipitated was pelleted at 10,000 rpm in a refrigerated microcentrifuge at 4°C (Hettich MICRO 24-48 R) for 30 minutes. Supernatant was removed carefully and the pellet was re-suspended in 0.01 M phosphate buffer (pH 7.0) containing 5 mM EDTA and 0.5 M urea. Again it was clarified by centrifugation at 5000 rpm for five minutes. The supernatant was taken and stored at -4°C and was used as antigen source for immunization of rabbits (Appendix XII).

3.7.2 Electron Microscopy

The standard procedure for obtaining a clarified viral concentrates (CVC) from BBrMV infected bract was as follows.

- Infected bract (1 g) was thoroughly homogenized in 2 ml of 0.1 M potassium phosphate buffer pH 7.5 containing 0.01 M Sodium di-ethyl dithiocarbomate (Na-DIECA), 0.1 per cent sodium sulphite and 2.0 per cent polyvinyl pyrrolidone (PVP). The homogenate was squeezed through cheese cloth (Appendix XIII).

- The filtrate was stirred with equal volume of n-butanol and chloroform mixture (1:1 v/v) for 10 minutes.
- The slurry was centrifuged at 10,000 rpm for 10 minutes and the pellet was discarded.
- PEG 6000 and sodium chloride (NaCl) was added to the supernatant (six per cent and 0.125 M respectively) and allowed to stand for 30 min under constant stirring.
- The mixture was centrifuged at 10,000 rpm for 10 minutes.
- The supernatant was discarded and the tubes were inverted on a dry filter paper to remove all the traces of liquid fraction.
- The pellet was re-suspended in 100 micro litre of the extraction buffer and centrifuged at low speed (5000 rpm) for five minutes.
- The pellet was discarded and the supernatant was used for coating grids for transmission electron microscopy (TEM)

A drop of CVC (30 µl) was placed on a piece of para film and collodion coated copper grid (400 mesh) was plotted on the drop of virus suspension, the film side for five to 10 min. The grid was then washed with 30 drops of distilled water followed by five drops of two per cent aqueous uranyl acetate and the grid was allowed to dry on a filter paper for few minutes. Each treatment was replicated three times. The grids were then observed under Jeol-JEM 100x transmission electron microscope.

3.7.3 Production of Antiserum against BBrMV

Antiserum was produced against BBrMV in a New Zealand white female rabbit (1 ½ years old) by giving five intra-muscular injections with the partially purified virus at weekly intervals. Before injection, virus preparation was emulsified with one ml of Freund's incomplete adjuvant (1:1 v/v) (Difco). Ten days after the last injection the rabbit was bled

through marginal ear vein. Blood was collected in a sterile test tube and was kept in a slightly slanting position for one hour at room temperature. After that the tube was kept without disturbing for overnight at 4°C. The clear serum was pipetted out and centrifuged at 5000 rpm for 30 minutes at 4°C. The supernatant was pipetted out using a micropipette and dispensed into 1.5 ml eppendorf tubes. A pinch of sodium azide was added to the clarified serum to prevent microbial contamination. The vials were stored under refrigerated condition.

3.7.3.1 Determination of Antibody Titre

Titre of the antiserum was determined by DAC-ELISA. The procedure described by Huguenot *et al.* (1992) was followed for the detection.

One gram of infected young leaf (without midrib) was homogenized in 5 ml of coating buffer (carbonate buffer) containing two per cent (w/v) PVP under chilled condition.

Healthy plant extract was prepared by using leaves of plants meristem cultured from healthy Nendran plants.

The homogenate was centrifuged at 5000 rpm for 10 minutes at 4°C. Samples were dispensed at the rate of 100 µl into Nunc immunological plates. The treatments were replicated thrice. After incubation for two hours at 37°C the wells were washed with Phosphate buffer saline–tween (PBS-T) three times each for a duration of three minutes. The plates were tapped on a blotting paper to remove PBS-T. Blocking was done with 100 µl of one per cent BSA for 30 minutes at 37°C. After incubation blocking agent was removed, plates were washed with PBS-T as before. The antibody raised in rabbit was diluted in healthy plant sap for cross absorption and 100 µl of each dilution was added to the well. Dilutions of 1:128, 1:256, 1:512, 1:1024 and 1:2048 were used for the determination of the titre of the developed antiserum. Three replications were maintained

for each treatment and incubated overnight at 4°C. The plates were washed with PBS-T and then treated with 100 µl of alkaline phosphatase conjugated goat-antirabbit immunoglobulin diluted in PBS-T Polyvinyl pyrrolidone Ovalbumin (PBS-TPO) (1:10,000 v/v) and incubated for two hours at 37°C. Wells were washed with PBS-T as before. The substrate p-nitro-phenyl phosphate (p-NPP) in diethanolamine buffer (1 mg per ml) was added to each well (100 µl per well) and incubated for one hour at 37°C. Reaction was stopped by adding 50 µl of four per cent NaOH. The absorbance was read at 405 nm in an ELISA reader (ECIL, MS 5608) (Appendix XIV).

3.7.4 Immunological Studies

3.7.4.1 Chloroplast Agglutination Test

A drop of virus infected sap squeezed through a muslin cloth was mixed with same amount of homologous antiserum with a tooth pick on a clean washed microscopic slide. Wherever virus was present chloroplasts in the sap aggregated together forming a precipitate which became very distinct in transmitted light or observed under a stereo binocular microscope for agglutination. If the reaction was delayed, the slide was incubated at 25°C for five minutes and gently warmed. Normal rabbit serum mixed with buffer served as control.

3.7.4.2 Microprecipitin test on Slides

Thirty micro litres of antiserum raised in rabbit and same quantity of BBrMV infected leaf extract in 0.1 M phosphate buffer (pH 7.0) were mixed on a microscopic slide. Antibody treated with healthy banana leaf extract served as control. The mixture was incubated at 25°C under high humidity for 20-45 minutes and examined under microscope.

3.7.4.3 Agar Gel Double Diffusion Test (Ouchterlony's Agar Gel Double Diffusion Test)

Double diffusion test was carried out as per the procedure of Ouchterlony (1962) with slight modification.

Cleared microscopic slides were used for conducting the test. Slides were pre-coated with 0.2 per cent fomvar dissolved in chloroform. Pre-treated slides were coated with thin layer of agarose gel at the rate of 2.5 ml agarose gel / slide. Then the slides were placed on a marked paper showing the position of wells at four equidistant positions at 40° angle around the centre point and two mm distance from the outer edge of the central well. Metallic tube of about 2 mm diameter was used to puncture the wells and using mild vacuum, wells were made on the punctured position. The central well of each slide received eight µl of the antiserum cross absorbed. The surrounding upper wells received eight µl of the antigen (i.e., infected leaf ground in PBS-TPO 1:2 w/v) whereas surrounding lower wells received healthy plant sap. Slides were incubated in humid condition in a glass jar and examined for the appearance of characteristic preciptin bands. Three replications were maintained.

After the development of precipitin bands the agarose gel was transferred to distilled water. After one hour the gel was treated with 0.85 per cent sodium chloride solution for 30 minutes. Then the gel was washed with distilled water, transferred to a clean slide and allowed to dry under a bell jar. Gel was stained with amido black (2 drops/slide) for one minute, washing of the gel with decolouriser was made until perfect bands were made visible (Appendix XV).

3.7.4.4 Enzyme Linked Immunosorbent Assay (ELISA)

As described above DAC-ELISA for the detection of BBrMV in the following samples was done by using the antiserum developed specific to BBrMV.

Samples used for the detection

- Young leaves of healthy meristem cultured plants
- Young leaves of plants meristem cultured from BBrMV infected suckers
- Pseudostem of plants meristem cultured from BBrMV infected suckers
- BBrMV infected leaf from the field
- BBrMV infected pseudostem from the field
- BBrMV infected bract from the field

3.7.4.5 Dot Immuno Binding Assay (DIBA) for the Detection of BBrMV

DIBA was carried out to detect BBrMV infected Nendran suckers using young leaves of BBrMV infected banana. The leaves of meristem cultured plants from healthy suckers were used as control.

Procedure

- Tissue was extracted in antigen extraction buffer (1:10 w/v) and expressed through cheese cloth
- 0.8 ml of expressed sap was taken in an eppendorf tube to which 0.4 ml chloroform was added
- The mixture was vortexed and centrifuged at 12,000 g for two minutes
- The clarified sap (upper aqueous layer) was mixed with antigen extraction buffer (1:4 ratio) and vortexed
- Nitrocellulose membrane (NCM) with squares of 1 x 1 cm was floated in Tris Buffer Saline (TBS) and air dried
- 10 µl of the sample was spotted to the centre of each square and allowed it to dry
- Treated NCM was immersed in blocking solution with gentle oscillation for one hour at room temperature

- NCM was rinsed in TBS for 10 minutes and incubated overnight at 4°C in crude antiserum diluted in TBS-Spray dried milk (SDM).
- NCM was again rinsed in TBS for 10 minutes and incubated for one hour at room temperature in secondary antibody (enzyme linked antirabbit Ig G diluted in TBS-SDM.
- After rinsing in TBS for 10 minutes NCM was incubated in substrate solution at room temperature in the dark
- After the colour development NCM was rinsed in fixing solution for 10 minutes and then air dried between Whatman filter paper sheets and stored (Appendix XVI).

3.8 MANAGEMENT

3.8.1 Screening for Resistance

The varieties grown at the Instructional Farm, Vellayani and germplasm collection at Banana Research Station (BRS), Kannara were screened for their reaction to BBrMV based on the symptoms under natural field conditions. Per cent disease incidence (DI) and per cent disease index (PDI) were recorded twice at six months intervals. Scoring was done using 0-4 scale as mentioned earlier. Based on the scoring per cent disease index was calculated.

3.8.2 Virus Elimination through Meristem Culture

3.8.2.1 Source of Explants

Infected Nendran suckers maintained in earthen pots under glass house condition were used for the study.

The nutrient medium developed by Murashige and Skoog (1962) was used as the basal medium.

Stock solutions of macro, micro, minor nutrients, iron and vitamins were prepared by dissolving adequate quantities of each chemical as per

Murashige and Skoog (1962). Stock solution of the growth regulator Benzyl Adenine (BA) was maintained separately (Appendix XVII).

3.8.2.2 Preparation of Sterile Tissue

The meristem portion separated from the infected suckers were thoroughly washed in tap water and were cut into handy sizes. These meristems were soaked in two per cent labolene solution for thirty minutes and again washed with distilled water (three times) to remove any trace of solution. Finally they were kept in sterilized water. These meristems were transferred to 0.08 per cent mercuric chloride for five minutes. The treated portions were washed in two changes of sterile distilled water. The above procedure was carried out in a laminar air flow chamber.

3.8.2.3 Dissection and Explant Preparation

Using sterile forceps the disinfected meristems were transferred to the stage of a dissection microscope. Viewing through the microscope meristem explants of about 3 mm size was removed using a sterile blade. The entire procedure was carried out in a laminar air flow chamber.

3.8.2.4 Inoculation of Explants

The meristem explants were inoculated into jam bottles having 15 ml of media in such a way that one third of the explant was inserted into the media.

3.8.2.5 Incubation of Cultures

Following excision and inoculation, the cultures were placed in an incubation room at 24 ± 4°C giving a photoperiod of 16 h.

3.8.2.6 Induction Media

Murashige and Skoog media (MS media) with BA at the rate of 5 mg l^{-1} was used for induction. A shoot 1-2 cm in length without roots developed from explant was used for regeneration.

3.8.2.7 Regeneration Media

Each shoot of about 1-2 cm length on the induction medium was then transferred to the regeneration medium. After the development of root and small shoot, the regenerated plants were transferred to small disposable cups containing sterile soil for establishment.

3.8.2.8 Hardening

Regenerated plants were planted in small disposable plastic cups having 1:1 v/v mixture of sterile sand and soil. The plants were covered with polythene bags with a hole for watering for one week. The covers were removed and the plantlets were exposed to full sunlight in insect proof condition.

The plants were observed daily for development of symptom. After proper hardening seedlings were transferred to earthen pots maintained in insect proof glasshouse and tested for the presence of BBrMV upto four months after planting. DAC-ELISA was used to index the plantlets.

3.9 STATISTICAL ANALYSIS

The data generated from the experiment were subjected to analysis of variance (ANOVA) after appropriate transformations wherever needed.

RESULTS

4. RESULTS

Banana bract mosaic virus (BBrMV) causing banana bract mosaic disease (BBrMD) locally known as Kokkan is a serious threat to banana production and is spreading fast at present in almost all the banana varieties cultivated in Kerala. A study was conducted on transmission, purification, characterization, pathophysiology, immunodetection of virus and management of the disease. Salient findings of the study are given below:

4.1 SURVEY

Survey on the incidence of BBrMV disease was carried out in the banana growing areas of Thiruvananthapuram district following a stratified two stage random sampling technique. The strata were taken as four taluks of the district *i.e.*, Nedumangadu, Neyyatinkara, Thiruvananthapuram and Chirayinkeezhu.

From each taluk three panchayaths were selected at random *i.e.*, Kilimanoor, Kattakkada, Peringamala (Nedumangadu), Thirupuram, Athiyannoor, Balaramapuram (Neyyatinkara), Malayinkeezhu, Pallichal, Venganoor, (Thiruvananthapuram) and Chirayinkeezhu, Kadakkavoor, Edava (Chirayinkeezhu).

From each of this selected panchayath five plots having a minimum of 100 plants were selected randomly. Varietal reaction and types of symptoms were observed. Data on disease incidence (DI) and per cent disease index (PDI) were recorded from each plot.

Scoring of the disease was done using a 0-4 scale and the PDI was calculated.

4.1.1 Disease Incidence

Out of the 60 plots surveyed nine plots were found healthy with no incidence of BBrMD. In the remaining 51 plots the PDI varied from 1 – 75. Majority of plots (35 nos.) were having PDI ranging from 1 - 25 while in 15 plots moderate DI was observed ranging from 26 - 50 per cent. Only one plot was having a higher DI in the range 51 – 75 (Table 2).

Table 2. Variation in per cent disease incidence of surveyed plots

Per cent disease incidence	Number
0	9
1 – 25	35
26 – 50	15
51 – 75	1
76 – 100	0

In the first observation in Nedumangadu taluk (T_1), the incidence in Peringamala panchayath (T_1P_3) was significantly less whereas that of Kattakada panchayath (T_1P_2) and Kilimanoor panchayath (T_1P_1) were on par. In Neyyattinkara taluk (T_2) the per cent incidence was statistically significant in all three panchayaths and the maximum was recorded in Balaramapuram panchayath (T_2P_3) and the least was in Athiyannoor panchayath (T_2P_2). In Thiruvananthapuram taluk (T_3) the incidence in the panchayaths Malayinkeezhu panchayath (T_3P_1) and Pallichal panchayath (T_3P_2) were on par whereas that of Venganoor (T_3P_3) was significantly low. In Chirayinkeezhu (T_4) taluk disease incidence in Chirayinkeezhu panchayath (T_4P_1) and Kadakkavoor panchayath (T_4P_2) were on par whereas no incidence was noted at Edava panchayath (T_4P_3) (Table 3).

Table 3. Per cent disease incidence of BBrMV in surveyed plots (Observation-I)

Sl. No.	Panchayath	Per cent disease incidence			
		Taluk			
		T_1	T_2	T_3	T_4
1	P_1	23.26	23.62	26.75	28.07
2	P_2	23.28	13.68	29.77	30.98
3	P_3	14.27	30.83	19.88	0.00
	Mean (Taluk)	20.28	22.71	25.47	19.69

CD (0.05) between Panchayaths within taluks = 6.54

Nedumangadu (T_1)	**Neyyattinkara (T_2)**	**Thiruvananthapuram (T_3)**	**Chirayinkeezhu (T_4)**
T_1P_1 Kilimanoor	T_2P_1 Thirupuram	T_3P_1 Malayinkeezhu	T_4P_1 Chirayinkeezhu
T_1P_2 Kattakkada	T_2P_2 Athiyannoor	T_3P_2 Pallichal	T_4P_2 Kadakkavoor
T_1P_3 Peringamala	T_2P_3 Balaramapuram	T_3P_3 Venganoor	T_4P_3 Edava

Statistical analysis of the data collected after six months showed that the disease incidence was increased in each plot. Among the 60 plots one to 25 per cent, 26 to 50 per cent, 51 to 75 per cent and 76 to 100 per cent disease incidence was observed in 24, 24, 8 and 4 plots respectively (Table 4). At this stage also there was no significant difference in disease incidence observed between the four taluks. It was found that disease incidence in all the three panchayaths were on par for the taluks T_1 and T_3 whereas for T_2 the same was statistically significant and it was maximum in Balaramapuram and least in Athiyannoor. In T_4 incidence in two panchayaths *i.e.*, Chirayinkeezhu and Kadakkavoor were on par whereas that in Edava was significantly low (Table 5).

Table 4 Variation in per cent disease incidence in surveyed plots

Per cent disease incidence	Number
0	0
1 – 25	24
26 – 50	24
51 – 75	8
76 – 100	4

Table 5. Per cent disease incidence of BBrMV in surveyed plots (Observation-II)

Sl. No.	Panchayath	Per cent disease incidence			
		Taluk			
		T_1	T_2	T_3	T_4
1	P_1	35.84	39.67	39.58	38.67
2	P_2	33.66	26.82	39.07	39.74
3	P_3	32.22	56.23	32.83	17.79
	Mean (Taluk)	33.91	40.91	37.16	32.07

CD (0.05) between Panchayaths within taluks = 9.68

Nedumangadu (T_1)	**Neyyattinkara (T_2)**	**Thiruvananthapuram (T_3)**	**Chirayinkeezhu (T_4)**
T_1P_1 Kilimanoor	T_2P_1 Thirupuram	T_3P_1 Malayinkeezhu	T_4P_1 Chirayinkeezhu
T_1P_2 Kattakkada	T_2P_2 Athiyannoor	T_3P_2 Pallichal	T_4P_2 Kadakkavoor
T_1P_3 Peringamala	T_2P_3 Balaramapuram	T_3P_3 Venganoor	T_4P_3 Edava

4.1.2 Per cent Disease Index (PDI)

Hundred plants in each plot were scored for BBrMV using 0 – 4 scale. PDI was calculated and presented in the Table 6. The results showed that the PDI between the four taluks were not statistically significant whereas it was significantly different between panchayaths. The PDI of BBrMV was nil in nine plots.

In T_1, PDI was maximum in Peringamala and was least in Kilimanoor. In T_2, T_3 and T_4 the maximum PDI was recorded in Balaramapuram, Pallichal and Kadakkavoor and the least in Athiyannoor, Venganoor and Edava respectively (Table 6).

Table 6. Per cent disease index of BBrMV in surveyed plots (Observation-I)

Sl. No.	Panchayath	Per cent disease index			
		Taluk			
		T_1	T_2	T_3	T_4
1	P_1	3.05	3.08	2.70	3.89
2	P_2	3.31	2.88	3.56	4.81
3	P_3	3.58	4.56	2.56	0.00
--	Mean (Taluk)	3.31	3.51	2.94	3.23

CD (0.05) between Panchayaths within taluks = 0.282

Nedumangadu (T_1)	Neyyattinkara (T_2)	Thiruvananthapuram (T_3)	Chirayinkeezhu (T_4)
T_1P_1 Kilimanoor	T_2P_1 Thirupuram	T_3P_1 Malayinkeezhu	T_4P_1 Chirayinkeezhu
T_1P_2 Kattakkada	T_2P_2 Athiyannoor	T_3P_2 Pallichal	T_4P_2 Kadakkavoor
T_1P_3 Peringamala	T_2P_3 Balaramapuram	T_3P_3 Venganoor	T_4P_3 Edava

Statistical analysis of the data collected six months after the first observation revealed that the incidence at three taluks were on par (*i.e.*, T_1, T_2

and T_4) whereas it was significantly different between T_2 and T_3. Per cent disease index between panchayaths of each taluk was statistically significant. For T_1, T_2, T_3 and T_4 the incidence was maximum in Peringamala, Balaramapuram, Pallichal and Kadakkavoor and the least was in Kilimanoor, Athiyanoor, Venganoor and Edava respectively (Table 7).

Table 7. Per cent disease index of BBrMV in surveyed plots (Observation-II)

Sl. No.	Panchayath	Per cent disease index			
		Taluk			
		T_1	T_2	T_3	T_4
1	P_1	3.54	4.84	3.93	4.82
2	P_2	4.15	3.75	4.31	6.85
3	P_3	4.94	6.91	3.29	3.66
	Mean (Taluk)	4.21	5.17	3.85	4.78

CD (0.05) between Panchayaths within taluks = 1.01

Nedumangadu (T_1)	**Neyyattinkara (T_2)**	**Thiruvananthapuram (T_3)**	**Chirayinkeezhu (T_4)**
T_1P_1 Kilimanoor	T_2P_1 Thirupuram	T_3P_1 Malayinkeezhu	T_4P_1 Chirayinkeezhu
T_1P_2 Kattakkada	T_2P_2 Athiyannoor	T_3P_2 Pallichal	T_4P_2 Kadakkavoor
T_1P_3 Peringamala	T_2P_3 Balaramapuram	T_3P_3 Venganoor	T_4P_3 Edava

4.1.3 Varietal Reaction

From the survey it was observed that none of the commonly cultivated varieties *i.e.*, Nendran, Robusta, Njalipoovan, Palayankodan, Red banana, Poovan and Vellakkappa were resistant to BBrMV (Table 8). If the infection occurs in the early stages of the plant growth, yield reduction was more in Robusta compared to other varieties.

Table 8 Reaction of different varieties of banana to BBrMV in the survey

Varieties	Number of plants observed	Number of plants infected	Per cent infection
Nendran	4000	3012	75.30
Robusta	600	332	55.33
Njalipoovan	200	79	39.50
Palayankodan	400	205	51.25
Red banana	750	600	80.00
Poovan	41	11	26.83
Vellakkappa	9	2	22.22

4.1.4 Symptomatology

Initial symptoms of BBrMD were characterized by the production of longitudinal irregular reddish streaks of varying sizes on pseudostem (Plate 1) and chlorosis of the leaf. As the disease progressed streaks become very prominent on the pseudostem, leaf petioles and on bracts (Plate 2). Most of the plants did not show foliar symptom (chlorosis) and if present were on younger leaves.

In some plants necrotic streaks were developed on pseudostem and leaf petioles (Plate 3). There was congested leaf arrangement in some of the plants observed and were travellers' palm like in appearance (Plate 4).

Small unmarketable bunches bearing small malformed fingers were produced in BBrMV infected plants (Plate 5). Early infection resulted in severe yield reduction.

4.2 TRANSMISSION

All of the transmission studies were carried out under glasshouse condition.

4.2.1 Transmission through Seed Material

Diseased and healthy suckers were planted separately in three seasons and observations were taken upto three months and the results

Plate 1. Longitudinal irregular reddish streaks

Plate 2. Spindle shaped streaks on bract

Plate 3. Necrotic streaks on pseudostem

Plate 4. Travellers' palm like appearance

Plate 5. Production of small malformed bunches due to BBrMV infection

were presented in Table 9. The result indicated that when infected suckers were used for planting 100 per cent transmission was recorded in all the three seasons, whereas for healthy suckers no disease incidence was recorded.

Table 9. Transmission of BBrMV through seed material under glasshouse condition

Seasons	Diseased			Healthy		
	Number of suckers planted	Number of suckers infected	Per cent infection	Number of suckers planted	Number of suckers infected	Per cent infection
August – November	20	20	100.00	20	0	0
December–March	20	20	100.00	20	0	0
April – July	20	20	100.00	20	0	0

4.2.2 Mechanical Transmission

4.2.2.1 Sap Inoculation by Leaf Rubbing

Results of sap transmission studies by leaf rubbing showed that BBrMV could not be transmitted by sap inoculation to banana eventhough different buffers at different pH were tried (Table 10).

Table 10. Mechanical transmission of BBrMV using different buffers

Buffers	pH	Number of Nendran plants inoculated	Number of Nendran plant infected
Sodium borate buffer (0.1M)	8.0	10	0
Potassium phosphate buffer (0.1 M) with and without sodium diethyl dithiocarbomate	7.2	10	0
Sodium phosphate buffer (0.1 M)	7.2	10	0
Tris buffer (0.1 M)	7.2	10	0
Citrate buffer (0.1 M)	7.2	10	0

Sap transmission study to transmit BBrMV from infected Nendran to local lesion hosts like *Nicotiana tabacum* and *Nicotiana benthamiana* also showed that sap transmission could not be achieved.

4.2.2.2 Sap Injection through Syringe

BBrMV infected sap was injected to healthy suckers and the plants were observed for three months. None of the plants developed the disease symptom.

4.2.2.3 Pin-prick Inoculation

Pin-pricks were made on spindle leaf of healthy suckers and extract containing BBrMV was applied on the injured area. Plants were maintained for three months for the symptom development. Results showed that the virus could not be transmitted by pin-prick inoculation.

4.2.2.4 Inoculation using Cutting Knives

It was observed that BBrMV was not transmitted through cutting knives.

4.2.3 Graft Transmission

Core grafting and root grafting also failed in transmitting BBrMV to healthy banana plants.

4.2.4 Insect Transmission

4.2.4.1 Collection and Rearing of Vector

The vectors *P. nigronervosa, A. gossypii* and *A. craccivora* were reared successfully and maintained in the glasshouse in suitable host species.

4.2.4.2 Transmission through Aphid Vectors

Insect transmission studies were conducted using *P. nigronervosa, A. gossypii* and *A. craccivora* and results are given in Table 11. The results showed that the aphid *P. nigronervosa* (Plate 6) alone was able to

Plate 6. ***Pentalonia nigronervosa*****, the aphid vector of BBrMV**

transmit the BBrMD (40 per cent) whereas *A. gossypii* and *A. craccivora* failed to transmit the disease.

Table 11. Transmission of BBrMV by different species of aphid

Sl. No.	Name of the aphid species	Number of plants inoculated	Number of plants Infected	Per cent transmission
1	*Pentalonia nigronervosa*	25	10	40
2	*Aphis craccivora*	25	0	0
3	*Aphis gossypii*	25	0	0

4.2.4.2.1 Effect of pre-acquisition fasting of *P. nigronervosa* on the efficiency of BBrMV transmission

The data presented in Table 12 reveals that pre-acquisition fasting period of one hour was found to be effective in the transmission of BBrMV (40 per cent). Further increase of fasting period neither increase the per cent transmission nor the efficiency of the vector to transmit the virus.

Table 12. Effect of pre-acquisition fasting of *P. nigronervosa* on the efficiency of transmission of BBrMV

Sl. No.	Pre-acquisition fasting period	Number of plants inoculated	Number of plants infected	Per cent transmission
1	No fasting	20	5	25
2	5 minutes	20	2	10
3	15 minutes	20	7	35
4	30 minutes	20	8	38
5	1 hour	25	12	40
6	2 hours	20	7	35

Acquisition feeding period - 30 minutes

Inoculation feeding period - 24 hour

Number of aphids per plant - 20

4.2.4.2.2 Acquisition Threshold of the Vector *Pentalonia nigronervosa*

The results of the experiment to find out the minimum period required for the vector to acquire the virus are presented in Table 13. The data showed that a short acquisition feeding period of one minute was sufficient for the aphid to become viruliferous. The optimum acquisition feeding period which gave the maximum per cent of infection (40 per cent) was found to be 30 minutes. As the acquisition feeding period was increased beyond 30 minutes the efficiency of vector to transmit the virus was reduced considerably. It was 28 per cent when the acquisition feeding period was one hour.

Table 13. Acquisition threshold of *P. nigronervosa* on the transmission of BBrMV

Sl. No.	Acquisition feeding period	Number of plants inoculated	Number of plants infected	Per cent transmission
1	One minute	20	3	15
2	15 minutes	20	6	30
3	30 minutes	25	10	40
4	45 minutes	25	9	36
5	1 hour	25	7	28

Pre-acquisition fasting period – 1 hour

Inoculation feeding period – 24 hour

Number of aphids per plant – 20

4.2.5 Soil Transmission

Results showed that none of the plants showed any symptoms of the disease even after three months (Table 14).

Table 14. Transmission of BBrMV through soil

Sl. No.	Soil type	Number of suckers planted	Number of suckers infected	Per cent plants infected
1	Soil from basins of BBrMV infected plants	15	0	0
2	Soil from disease free areas	15	0	0

4.3 PATHOPHYSIOLOGY

Changes in total carbohydrates, chlorophyll, total phenol, OD-phenol, protein and activity of defence related enzymes *viz.*, perdoxidase, polyphenol oxidase and phenylalanine ammonia lyase were studied at four stages *i.e.*, 3 MAP, 6 MAP, flag leaf stage and bract (flowering stage).

Since there was considerable reduction in fruit size due to BBrMV infection bioassay of endogenous growth substances *viz.*, cytokinin, auxin and giberellic acid were also conducted.

4.3.1 Total Carbohydrate

The total carbohydrate content of healthy and BBrMV infected banana plants were estimated and the results are presented in Table 15. It was observed that the total carbohydrate content of BBrMV infected banana leaves were decreased to varying degrees at 3 MAP, 6 MAP and flag leaf stages (*i.e.*, per cent decrease over healthy was 49.75, 64.78, 35.68 respectively) when compared to healthy leaves. Whereas the content of the same was increased in the infected bract (per cent increase over healthy was 19.51). The differences in total carbohydrate content were found to be statistically significant at all stages of analysis.

Table 15. Changes in total carbohydrate content (mg/g) of banana leaves as well as bract in response to BBrMV infection*

Stages of the plant	Healthy (µg/g)	Infected (µg/g)	Per cent increase (+) or decrease (-) over healthy
3 MAP	40.50	20.35	-49.75
6 MAP	88.75	31.25	-64.78
Flag leaf	53.25	34.25	-35.68
Bract	20.50	24.50	+19.51

*Mean of four replication

CD 0.05 level

Period x conditions 2.16

4.3.2 Chlorophyll

Chlorophyll was estimated by the method described by Arnon (1949) and the results are presented in the Table 16.

The total chlorophyll content was found to be less in the leaves of BBrMV infected plants at all stages when compared to that of healthy plants. The maximum reduction in total chlorophyll content (79.45 per cent) was at the flag leaf stage (Table 16). The differences in total chlorophyll content were significant at all stages of analysis.

The results also indicated that BBrMV infection caused significant reduction in the content of chlorophyll *a* and chlorophyll *b* compared to healthy leaves at all stages of analysis. It was found that for chlorophyll *a* the maximum per cent reduction over healthy (66.7 per cent) was at six months after planting and for chlorophyll *b* the highest reduction of 89.66 per cent over healthy was observed at three months after planting (Table 16).

Table 16 Changes in chlorophyll content (mg/g leaf tissue) in the leaves of banana plants in response to BBrMV infection

Stages of the plant	Total chlorophyll			Chlorophyll *a*			Chlorophyll *b*		
	Healthy	Infected	Per cent decrease (-) over healthy	Healthy	Infected	Per cent decrease (-) over healthy	Healthy	Infected	Per cent decrease (-) over healthy
3 MAP	0.63	0.23	-63.49	0.31	0.21	-32.30	0.29	0.03	-89.66
6 MAP	1.02	0.34	-66.66	0.75	0.25	-66.70	0.27	0.09	-66.66
Flag leaf	0.73	0.15	-79.45	0.51	0.10	-62.75	0.27	0.04	-85.19
Bract	0.52	0.19	-63.46	0.35	0.11	-51.43	0.14	0.07	-7.14
	CD 0.05 level			CD 0.05 level			CD 0.05 level		
	Period x condition 0.02			Period x condition 0.01			Period x condition 0.01		

4.3.3 Total Phenol

The total phenolic content was estimated according to the procedure given by Bray and Thorpe (1954) and the results are presented in Table 17.

The BBrMV infected plants had higher total phenol content at six MAP and flag leaf stage (per cent increase over healthy was 0.08 and 0.20 respectively) and it was less at three MAP and bract stage (per cent decrease over healthy was 0.23 and 0.13 respectively). The differences in phenol content were statistically significant at all stages of analysis.

Table 17. Total phenolic content (µg/g tissue) in the leaves and bract of healthy and BBrMV infected plants*

Stages of the plant	Healthy (µg/g)	Infected (µg/g)	Per cent increase (+) or decrease (-) over healthy
3 MAP	6.69 (2.77)	5.13 (2.47)	-0.23
6 MAP	4.51 (2.35)	4.88 (2.43)	0.08
Flag leaf	6.51 (2.74)	7.83 (2.97)	0.20
Bract	5.11 (2.47)	4.45 (2.33)	-0.13

CD 0.05 level

Period x condition 0.014 *Mean of four replication

Data in parenthesis are transformed means

4.3.4 Estimation of Ortho Dihydroxy Phenol (OD-phenol)

The OD-phenol content was significantly higher in BBrMV infected banana plants at all stages of analysis when compared to healthy except at six MAP. At six MAP OD-phenol content of both healthy and infected samples were on par (0.046 and 0.047). The OD-phenol content was maximum in the infected bract (0.049) (Table 18).

Table 18 Changes in the OD-phenol content (μg/g tissue) of banana plants in response to BBrMV infection*

Treatment	Healthy	Infected	Per cent increase over healthy
3 MAP	0.043	0.046	6.98
6 MAP	0.046	0.047	2.17
Flag leaf	0.043	0.047	9.30
Bract	0.047	0.049	4.25

CD 0.05 level

Period x condition 0.002 *Mean of four replications

4.3.5 Protein

Estimation of protein was carried out as per the procedure given by Bradford (1976) and the results are presented in Table 19. The results indicated that the infection of plants with BBrMV caused a significant increase in protein content at all stages of growth when compared to healthy. The highest protein content was noticed at three MAP (32.30 per cent increase over healthy).

In healthy as well as infected samples there was a decreasing trend in protein content at different growth stages till bunch formation.

Table 19. Changes in total soluble protein content (mg/g) of banana in response to BBrMV infection*

Stages of plant	Healthy	Infected	Per cent increase over healthy
3 MAP	16.25	21.50	32.30
6 MAP	10.75	12.00	11.63
Flag leaf	8.00	9.50	18.75
Bract	9.75	12.50	28.21

CD 0.05 level

Period x Condition 1.24 *Mean of four replications

4.3.6 Defense Related Enzymes

4.3.6.1 Estimation of Peroxidase (PO) Activity

Compared to healthy samples there was a progressive increase in PO activity of infected samples at all stages of analysis and the per cent increase over healthy was maximum in the infected bract (730.80). The differences in PO activity between healthy and diseased samples were statistically significant (Table 20).

Table 20. Effect of BBrMV infection on peroxidase activity per minute per gram tissue of banana plants*

Treatment	Change in OD value g^{-1} min^{-1}		
	Healthy	Infected	Per cent increase over healthy
3 MAP	0.015	0.050	233.00
6 MAP	0.043	0.103	139.50
Flag leaf	0.053	0.113	113.20
Bract	0.013	0.108	730.80

CD 0.05 level

Condition x Period 0.11 *Mean of four replications

4.3.6.2 Estimation of Polyphenol Oxidase (PPO) Activity

The differences in PPO activity were statistically significant in healthy and BBrMV infected plants at all stages of analysis.

Compared to healthy samples BBrMV infected samples showed significantly higher PPO activity at all stages of analysis. The per cent increase over healthy was maximum at six MAP (192.0) (Table 21).

Table 21. Effect of BBrMV infection on PPO activity in banana plants*

Stages of analysis	Change in OD value g^{-1} min^{-1}		Per cent increase over healthy
	Healthy	Infected	
3 MAP	0.205	0.255	24.39
6 MAP	0.125	0.365	192.0
Flag leaf	0.205	0.308	50.24
Bract	0.163	0.185	13.47

CD 0.05 level

Conditions x Period 0.011 *Mean of four replication

4.3.6.3 Estimation of Phenylalanine Ammonia Lyase (PAL) Activity

The diseased plant parts had significantly higher PAL activity compared to healthy at all stages of analyses except at six MAP. At six MAP (2.08) PAL activity of healthy as well as infected plants were on par. The highest activity of PAL in BBrMV infected sample (5.54) was at bract stage (Table 22).

Table 22. Changes in PAL activity of banana plants (μg g^{-1}) in response to BBrMV infection*

Stages of plant	Healthy	Infected	Per cent increase over healthy
3 MAP	1.58	2.89	82.91
6 MAP	2.04	2.08	1.96
Flag leaf	2.07	2.46	18.84
Bract	2.87	5.54	93.03

C.D 0.05 level

Conditions x Period 0.197 *Mean of four replication

4.4 ELECTROPHORETIC ANALYSIS OF PROTEINS

4.4.1 Characterization of Protein by (Sodium Dodecyl Sulphate-Polyacrylamide Gel Electrophoresis) SDS-PAGE

SDS-PAGE was done for proteins following the protocol developed by Laemmeli (1970).

The protein extracted from young leaves of healthy as well as BBrMV infected Nendran plants revealed the presence of extra protein in infected samples. Three new major protein bands with estimated sizes of 31, 32 and 39 kDa (size estimate was carried out by comparison with Geneis low range protein molecular markers (PMW-L) were visible on Coomassie brilliant blue stained SDS polyacrylamide gels. These bands were absent from corresponding healthy preparations. Molecular weight of the extra bands revealed the presence of extracellular protein (that of BBrMV) in infected samples (Plate 7).

4.5 ELECTROPHORETIC ANALYSIS OF ISOZYME

Native polyacrylamide gel electrophoresis was carried out for isozyme analysis of polyphenol oxidase (PPO) and peroxidase (PO) to find out the variation in isozyme content of healthy and BBrMV infected Nendran plants. The relative mobility (Rm) value of each band was calculated and Rm was represented as Zymogram. Based on this, it was concluded that between healthy as well as BBrMV infected plants there was significant difference in isozyme production.

The PPO activity in both healthy as well as BBrMV infected plants produced single band with Rm value of 0.65. But in BBrMV infected sample the expression of PPO was more prominent compared to healthy (Plate 8).

The results presented in Plate 9 showed that healthy as well as BBrMV infected samples exhibited a distinct polymorphism in the case of PO. The BBrMV infected samples showed the presence of a definite

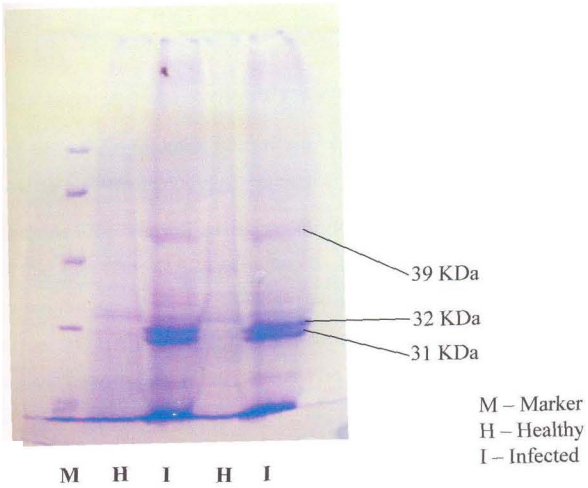


Plate 7. SDS-PAGE analysis of healthy as well as BBrMV infected samples

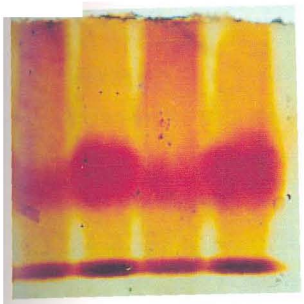


Plate 8. **Over expression of PPO in BBrMV infected banana plant**

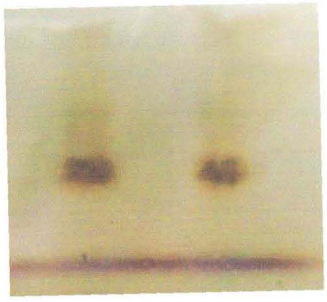


Plate 9. Induction of PO in BBrMV infected banana plants

single band with Rm value of 0.63 whereas this was absent in healthy samples.

4.6 BIOASSAY OF ENDOGENOUS GROWTH REGULATORS

Bioassay of growth regulators *i.e.*, auxin, cytokinin and gibberlic acid was carried out as per the protocol of Hansen *et al.* (1984).

For this study healthy as well as BBrMV infected fruits were used. The data revealed that the auxin (IAA) content was higher in healthy fruits (10^{-3} M IAA) compared to that of infected fruits (10^{-4} M IAA). For cytokinin as well as gibberlic acid (GA) the same trend was noticed. Cytokinin content of healthy Nendran fruits was found to be 10^{-6} M whereas that of BBrMV infected fruit was 10^{-7} M. In the case of gibberlic acid healthy samples showed presence of higher GA content (10^{-7} M) compared to infected ones (10^{-8} M). From these analysis all of the three growth regulators estimated were found to be low in BBrMV infected fruits compared to healthy.

4.7 CHARACTERIZATION

4.7.1 Purification of BBrMV

Partial purification of BBrMV was successfully done as per the procedure of Khurana *et al.* (1987). The virus was purified from the young banana leaves collected from the BBrMV infected plants maintained in the glasshouse.

4.7.2 Electron Microscopy

Electron microscopic studies by leaf dip method revealed that the virus particles were long flexuous rods. The size ranged from 700-900 nm x 10-15 nm with an average of 725 nm x 12 nm (Plate 10).

4.7.3 Production of Antiserum Against BBrMV

Antiserum was produced in New Zealand white rabbit against BBrMV by giving five intramuscular injections at weekly intervals with

Leaf dip method

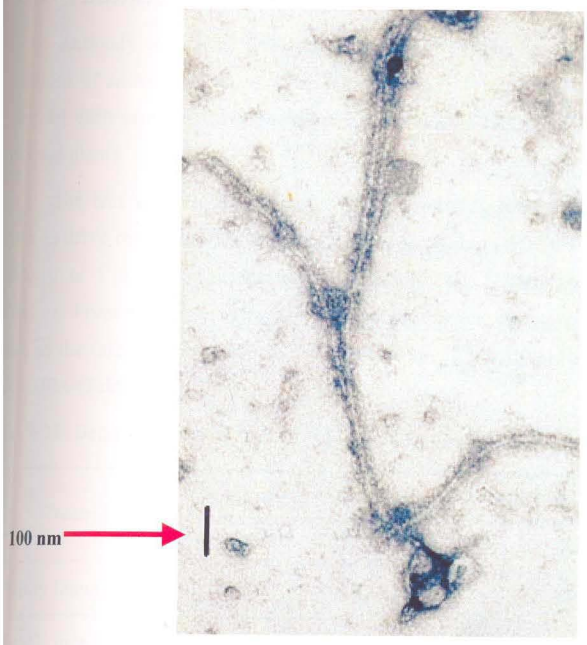


Plate 10. Flexuous rod shaped BBrMV viewed through an electron microscope

the partially purified virus with an equal volume of Freund's incomplete adjuvant. Antiserum was separated from the blood collected ten days after the last injection.

4.7.3.1 Determination of Antibody Titre

Antibody titre was determined by DAC-ELISA method. Different dilutions of antiserum *viz.*, 1:128, 1:256, 1:512, 1:1024 and 1:2048 were used for each samples from pseudostem and young leaves of systemically infected plants.

The leaf samples and samples from pseudostem of infected plants gave positive reaction with antisera produced against BBrMV at a higher dilution of 1 : 1024 whereas higher dilution *i.e.*, 1:2048 gave negative reaction. From the observation it was observed that the infected leaf as well as the infected pseudostem the BBrMV specific antiserum had a titre of 1:1024 (Table 23).

Table 23. Determination of titre of the antibody developed against BBrMV

Treatment	Absorbance at 405 nm				
	1 : 128	1 : 256	1 : 512	1 : 1024	1 : 2048
Healthy leaves	0.56	0.38	0.37	0.48	0.40
BBrMV infected leaf	0.85	0.71	1.28	1.31	0.91
Healthy pseudostem	0.58	0.41	0.47	0.45	0.50
BBrMV infected pseudostem	0.84	0.90	1.75	1.57	0.99

4.7.4 Immunological Studies

4.7.4.1 Chloroplast Agglutination Test

Wherever the BBrMV infected sap was allowed to react with antiserum produced against the virus, chloroplasts in the sap was

aggregated together forming a precipitate. This type of aggregation was not observed in healthy plant extract. This test was found successful for virus detection from crude sap of banana infected with BBrMV.

4.7.4.2 Microprecipitin Test

This test was also found to be successful for detection of BBrMV in crude sap. When the infected plant extract was allowed to react with BBrMV specific antiserum a white precipitate was formed on slides whereas this was absent for healthy control.

4.7.4.3 Agar Gel Double Diffusion Test (Ouchterlony's Agar Double Diffusion Test)

Agar gel double diffusion test was successful in detecting BBrMV in the crude sap of systemically infected banana. When extract of infected sample was placed in the surrounding wells and BBrMV specific antiserum in the central well, the positive sample showed the presence of a white band between the wells in the experiment (Plate 11).

4.7.4.4 ELISA (Enzyme Linked Immunosorbent Assay)

Antibody produced against partially purified BBrMV was used for ELISA. Samples were collected from both healthy and infected young leaf, pseudostem and bract. Titre of the antiserum used for the study was 1:512. The absorbance was measured at 405 nm in an ELISA reader (ECIL, MS 5608). The results of the experiment (Table 24 and Plate 12) revealed that the BBrMV specific antiserum gave high reactivity towards the virus isolate. Among the samples from different parts of infected plant, the bract showed highest absorbance (1.16) followed by pseudostem (0.62). For infected leaf the value was 0.62 whereas for healthy sample it was least (0.41).

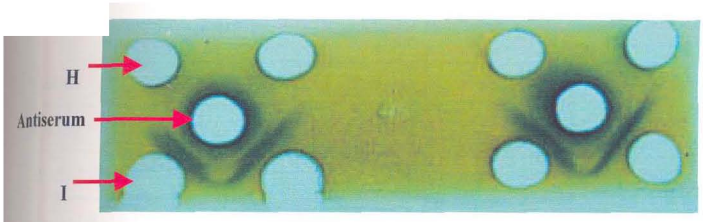
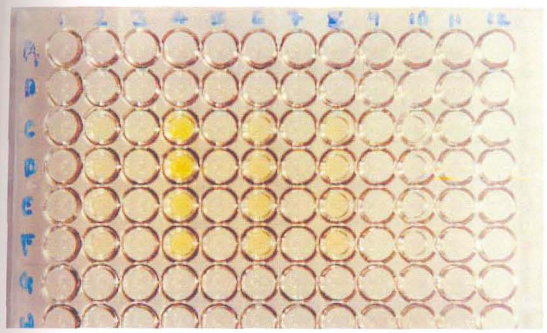


Plate 11. Ouchterlony's agar gel double diffusion test



H – Healthy,
Br – Bract,
PS – Pseudostem,
L – Leaf,
BC – Buffer control

H Br PS L BC

Plate 12. Reaction of different parts of banana infected by BBrMV to developed antiserum in DAC-ELISA

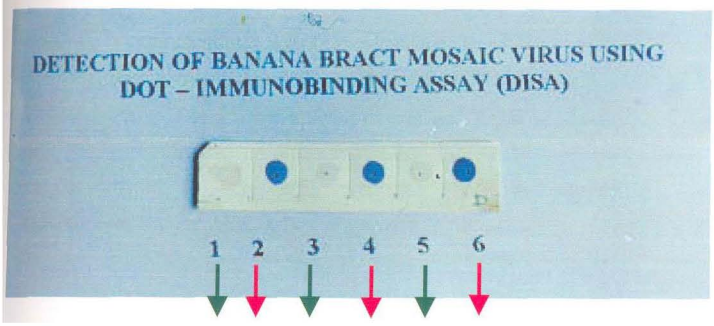


Plate 13. BBrMV infected samples detected by DIBA

Table 24 Reaction of different parts of banana infected by BBrMV to developed antiserum in DAC-ELISA

Sample	Absorbance at 405
Healthy	0.41
Infected bract	1.16
Infected pseudostem	0.62
Infected leaf	0.62

4.7.4.5 Dot Immunobinding Assay (DIBA)

DIBA was conducted on nitrocellulose membrane to detect the virus in BBrMV infected samples. The polyclonal antibody developed against BBrMV was used for the study. Infected leaf samples showed purple coloured spots in the NCM which showed that these samples contained BBrMV whereas the same spots were absent for healthy control (Plate 13).

4.8 MANAGEMENT OF THE DISEASE

4.8.1 Screening for Resistance

Banana varieties grown at the Instructional Farm, Vellayani and germplasm collection at the banana research station, Kannara were examined periodically for their reaction to BBrMV.

Major symptoms of BBrMV observed in the germplasm were chlorosis of young leaves, reddish or necrotic streaks on pseudostem, leaf petiole and bracts and in some cases travellers' palm appearance. The plants that were affected in the early stage by BBrMV showed severe reduction in yield. In some cases symptoms were seen only on suckers whereas mother plants appeared to be healthy.

Data collected from Kannara (Table 25) revealed that the accessions with 'A' genome was comparatively resistant to BBrMV compared to

those with 'B' genome. Even for those with 'B' genome the disease incidence recorded was very low. In Kannara, 180 accessions were screened for resistance and found that 162 accessions were seen free from any characteristic symptom of BBrMV.

Table 25 Observations on DI and PDI of Germplasm Collection at Kannara

Sl. No.	Accessions	3 MAP		6 MAP		Before bunching		After bunching	
		DI	PDI	DI	PDI	DI	PDI	DI	PDI
1	Pisanglin (AA)	0	0	0	0	0	0	0	0
2	Namrai (AA)	0	0	0	0	0	0	0	0
3	Sikuzani (AA)	0	0	0	0	0	0	0	0
4	Cultivar Rose (AA)	0	0	0	0	0	0	0	0
5	Musa acuminata (AA)	0	0	0	0	0	0	0	0
6	Pisang berlin (AA)	0	0	0	0	0	0	0	0
7	Pisang juaribuaga (AA)	0	0	0	0	0	0	0	0
8	Niyarmsik (AA)	0	0	0	0	0	0	0	0
9	Matti (AA)	0	0	0	0	0	0	0	0
10	Tongat (AA)	0	0	0	0	0	0	0	0
11	Kadali (AA)	0	0	0	0	0	0	0	0
12	Sanna Chenkadali (AA)	0	0	0	0	0	0	0	0
13	Erachi Vazha (AA)	0	0	0	0	0	0	0	0
14	Dwarf Cavendish (AAA)	0	0	0	0	0	0	0	0
15	Barsai (AAA)	0	0	0	0	0	0	0	0
16	Moris (AAA)	0	0	0	0	0	0	0	0
17	Petit Naine (AAA)	0	0	0	0	0	0	0	0
18	Robusta (AAA)	0	0	0	0	0	0	0	0
19	Pedda pacha (AAA)	0	0	0	0	0	0	0	0
20	Culcutta-4 (AAA)	0	0	0	0	0	0	0	0

21	Mons Mari (AAA)	0	0	0	0	0	0	0	0
22	Grand naine (AAA)	0	0	0	0	0	0	0	0
23	High gate (AAA)	0	0	0	0	0	0	0	0
24	Yangamb KM-5 (AAA)	0	0	0	0	0	0	0	0
25	Amrit Sagar (AAA)	0	0	0	0	0	0	0	0
26	Chakkara Kali (AAA)	0	0	0	0	0	0	0	0
27	Lakkattan (AAA)	0	0	0	0	0	0	0	0
28	Sappulan annamala (AAA)	0	0	0	0	0	0	0	0
29	Gros Michel (AAA)	0	0	0	0	0	0	0	0
30	Namkanika (AAA)	0	0	0	0	0	0	0	0
31	Nakitemp (AAA)	0	0	0	0	0	0	0	0
32	Wather (AAA)	0	0	0	0	0	0	0	0
33	Manoranjitham (AAA)	0	0	0	0	3	20	13	20
34	Karivazha (AAA)	0	0	0	0	0	0	0	0
35	Chenkadali (AAA)	0	0	0	0	0	0	0	0
36	Pachakappa (AAA)	0	0	0	0	0	0	0	0
37	Anamalu (AAA)	0	0	0	0	0	0	0	0
38	KNR mutant (AAA)	0	0	0	0	0	0	0	0
39	SH-34441 (AAAA)	0	0	0	0	0	0	0	0
40	SH-34369 (AAAA)	0	0	0	0	0	0	0	0
41	Boldles Alta Fort (AAAA)	0	0	0	0	0	0	0	0
42	Anaikomban (AB)	10	3	35	25	48	40	60	45
43	Kunnan (AB)	0	0	0	0	20	10	20	15
44	Valiya Kunnan (AB)	25	10	40	17	60	40	65	40
45	Adukkan (AB)	0	0	0	0	0	0	0	0
46	Poomgalli (AB)	0	0	0	0	0	0	0	0
47	Poomkanna kadali (AB)	0	0	0	0	0	0	0	0
48	Adakka Kunnan (AB)	0	0	0	0	0	0	0	0

49	Nattu poovan (AB)	0	0	0	0	0	0	0	0
50	Agniswar (AB)	0	0	0	0	0	0	0	0
51	Padali Moongil (AB)	0	0	0	0	0	0	0	0
52	Njalipoovan (AB)	0	0	0	0	0	0	20	5
53	Nani poovan (AB)	0	0	20	10	25	15	35	20
54	Rasakadali (AB)	0	0	0	0	40	30	75	80
55	Rasagalli (AB)	0	0	0	0	0	0	0	0
56	Pachabale (AB)	0	0	0	0	0	0	0	0
57	Nalla Bontha (AB)	0	0	0	0	0	0	0	0
58	Kalyan bale (AB)	0	0	0	0	0	0	0	0
59	Velliputtu bale (AB)	0	0	0	0	0	0	0	0
60	Safed Velchi (AB)	0	0	0	0	0	0	0	0
61	ICNR mutant (AB)	0	0	0	0	0	0	0	0
62	Pathabontha batheesa (AB)	0	0	0	0	0	0	0	0
63	Kodappanilla kunnan (AAB)	0	0	0	0	0	0	0	0
64	PV 03-44 (AAB)	0	0	0	0	0	0	0	0
65	Thiruvananthapuram (AAB)	0	0	0	0	0	0	0	0
66	Mottapoovan (AAB)	0	0	0	0	4	20	4	20
67	Pisang Ceylon (AAB)	0	0	0	0	0	0	0	0
68	PKKNR (AAB)	0	0	0	0	0	0	0	0
69	Chandrabale (AAB)	25	10	40	25	75	45	100	60
70	Kari bale (AAB)	30	3.5	50	8.9	75	80	100	69
71	BRS-1 (AAB)	0	0	0	0	0	0	0	0
72	BRS-2 (AAB)	0	0	0	0	0	0	0	0
73	Nenthra kali (AAB)	0	0	0	0	0	0	0	0
74	H-3 (AAB)	0	0	0	0	0	0	0	0
75	Karivazha (AAB)	0	0	0	0	1	10	1	12
76	H-4 (AAB)	0	0	0	0	0	0	0	0

77	Chinali (AAB)	0	0	0	0	0	0	0	0
78	Karimkadali (AAB)	0	0	0	0	0	0	0	0
79	Kali bow (AAB)	0	0	0	0	0	0	0	0
80	Pachottan (AAB)	0	0	0	0	0	0	0	0
81	Dudh Sagar (AAB)	0	0	0	0	0	0	0	0
82	Pisang Seribu (ABB)	0	0	0	0	40	22.5	45	30
83	Sugandhi (AAB)	0	0	0	0	0	0	0	0
84	Pisangkela (AAB)	0	0	0	0	0	0	0	0
85	Popoalu (AAB)	0	0	0	0	0	0	0	0
86	Mysore Ethan (AAB)	0	0	0	0	0	0	0	0
87	Krishna vazha (AAB)	0	0	0	0	0	0	0	0
88	Nendran (AAB)	20	5	40	10	60	15	60	20
89	Krishna vazha (AAB)	0	0	0	0	0	0	0	0
90	Zanzibar (AAB)	0	0	0	0	0	0	0	0
91	Big Ehanga (AAB)	0	0	0	0	0	0	0	0
92	Virupakshi (AAB)	0	0	0	0	0	0	0	0
93	Myndoli (AAB)	0	0	0	0	0	0	0	0
94	Vannan (AAB)	0	0	0	0	44	22	49	25
95	Ladies Finger (AAB)	0	0	0	0	20	10	20	15
96	Kali (AAB)	0	0	0	0	0	0	0	0
97	Thenkali (AAB)	10	5	15	9.6	18	10	20	12
98	Nendra Padathi (AAB)	0	0	0	0	0	0	0	0
99	Mannan (AAB)	0	0	0	0	0	0	0	0
100	Cheena Bale (AAB)	0	0	0	0	0	0	0	0
101	Kali Bale (AAB)	0	0	0	0	0	0	1	4
102	Enna banian (AAB)	0	0	0	0	0	0	0	0
103	CO-1 (AAB)	2	3.1	3	3.5	15	5.0	65	15
104	Padathi (AAB)	0	0	0	0	0	0	0	0

105	Pachanadan (AAB)	0	0	0	0	20	8.0	50	25
106	Malakali (AAB)	0	0	0	0	0	0	0	0
107	Charakali (AAB)	0	0	0	0	0	0	0	0
108	Atrasingan (AAB)	0	0	0	0	0	0	0	0
109	Malavazha (AAB)	0	0	0	0	0	0	0	0
110	Kaliagali (AAB)	0	0	5	3.5	5	4.0	5	4.5
111	Navaral (AAB)	0	0	0	0	0	0	0	0
112	Velipadathi (AAB)	0	0	0	0	0	0	0	0
113	Red jassira (AAB)	0	0	0	0	0	0	0	0
114	Charapadathi (AAB)	0	0	0	0	0	0	0	0
115	Sawai (AAB)	0	0	0	0	0	0	0	0
116	Peyan (AAB)	0	0	0	0	0	0	0	0
117	Perumpadali (AAB)	0	0	0	0	0	0	0	0
118	Thekkan Thulladan (AAB)	0	0	0	0	0	0	0	0
119	Pevazha (AAB)	0	0	0	0	0	0	0	0
120	Amrithapani (AAB)	0	0	0	0	0	0	0	0
121	Marthoman (AAB)	0	0	0	0	0	0	0	0
122	Valiya poovan (AAB)	0	0	0	0	0	0	0	0
123	Malbhog (AAB)	0	0	0	0	0	0	0	0
124	Madhuraga (AAB)	0	0	0	0	0	0	0	0
125	Poovan KNR (AAB)	0	0	0	0	0	0	0	0
126	Birkhel (AAB)	0	0	0	0	0	0	0	0
127	Pisang Gaya (AAB)	0	0	1	2.3	8	3.7	10	20
128	Nedu Nendran (AAB)	0	0	0	0	0	0	0	0
129	Sirumali (AAB)	0	0	0	0	0	0	0	0
130	Enikanban (AAB)	0	0	0	0	0	0	0	0
131	Tulsi malpig (AAB)	0	0	0	0	12	3	20	7.5
132	Nendrakkali (AAB)	0	0	0	0	0	0	0	0

133	Kanchikela (AAB)	0	0	0	0	0	0	0	0
134	Chakkia (AAB)	0	0	0	0	0	0	0	0
135	Bagnan (AAB)	0	0	0	0	0	0	0	0
136	Monthan – 1 (AAB)	0	0	0	0	0	0	0	0
137	Erodeka (AAB)	0	0	0	0	0	0	0	0
138	Bluggoe (AAB)	0	0	0	0	0	0	0	0
139	Pisang mas (AAB)	0	0	0	0	0	0	0	0
140	Cambi (ABB)	0	0	0	0	0	0	0	0
141	Pancha bontha bather (AAB)	0	0	0	0	0	0	0	0
142	Manja vazha (AAB)	0	0	0	0	0	0	0	0
143	Raja bale (AAB)	0	0	0	0	0	0	0	0
144	Anakomban (AAB)	0	0	0	0	0	0	0	0
145	Bainsa (AAB)	0	0	0	0	0	0	0	0
146	Monthan (AAB)	0	0	0	0	0	0	0	0
147	Chetty (AAB)	0	0	0	0	0	0	0	0
148	FHIA-1 (AAAB)	0	0	0	0	0	4	15	6.4
149	PA 03-22 (AAAB)	0	0	0	0	0	0	0	0
150	Sakai (ABB)	0	0	0	0	0	0	0	0
151	Buditu bontha batheesa (ABB)	0	0	0	0	0	0	0	0
152	Dole (ABB)	0	0	0	0	0	0	0	0
153	Beula (ABB)	0	0	0	0	0	0	0	0
154	Gauria (ABB)	0	0	0	0	0	0	0	0
155	Govakkai (ABB)	0	0	0	0	0	0	0	0
156	Alukkel (ABB)	0	0	0	0	0	0	0	0
157	Bersain (ABB)	0	0	0	0	0	0	0	0
158	Kostha bontha (ABB)	0	0	0	0	0	0	0	0
159	Chinia (ABB)	0	0	0	0	0	0	0	0
160	Octoman (ABB)	0	0	0	0	0	0	0	0

161	KNR 2175 (ABB)	0	0	0	0	0	0	0	0
162	Puloor (ABB)	0	0	0	0	0	0	0	0
163	Raja vazha (ABB)	0	0	0	0	0	0	0	0
164	Dakshin Sagar (ABB)	0	0	0	0	0	0	0	0
165	Chirapunchi (ABB)	0	0	0	0	0	0	0	0
166	Jurmani Kanthali (ABB)	0	0	0	0	0	0	0	0
167	Vellapalayankodan (ABB)	0	0	0	0	0	0	0	0
168	Nattuvazha (ABB)	0	0	0	0	0	0	0	0
169	Neyvannan (ABB)	0	0	0	0	0	0	0	0
170	Kallumonthan (ABB)	0	0	0	0	0	0	0	0
171	Peykunnan (ABB)	0	0	0	0	0	0	0	0
172	Sambrani monthan (ABB)	0	0	0	0	0	0	0	0
173	Bibutia (ABB)	0	0	0	0	0	0	0	0
174	Boot-Bale (AABB)	0	0	0	0	0	0	0	0
175	Hybrid sawai (AABB)	0	0	0	0	0	0	0	0
176	Malamonthan (BB)	0	0	0	0	0	0	0	0
177	Klue Parot (BBB)	0	0	0	0	0	0	0	0
178	Pidimonthan	0	0	0	0	0	0	0	0
179	*Musa ornata*	0	0	0	0	0	0	0	0
180	H_5 (*M. ornata* + Pisang linin)	0	0	0	0	0	0	0	0

MAP – Months after planting

DI – Per cent disease incidence PDI – Per cent disease index

Observations on germplasm collection at Vellayani also showed that eventhough accessions with 'A' genome showed susceptibility to BBrMV (about 30 per cent) accessions with 'B' genome were more susceptible to the disease (56 per cent). Here commonly cultivated varieties like Robusta, Chuvannakappa, Vellakappa, Palayankodan, Njalipoovan were found to be infected by BBrMV (Table 26).

Table 26 Screening of germplasm collection at Instructional Farm, Vellayani

Sl. No.	Accessions	3 MAP		6 MAP		BB	
		DI	PDI	DI	PDI	DI	PDI
1	Sanna Chenkadali (AA)	0	0	0	0	0	0
2	Pisanglin (AA)	0	0	0	0	0	0
3	Matti (AA)	0	0	0	0	0	0
4	Matti (AA)	0	0	0	0	0	0
5	Robusta (AAA)	10	8.7	85	40	100	75
6	Amrit (AAA)	0	0	0	0	0	0
7	Monsmori (AAA)	0	0	0	0	0	0
8	Chuvanna kappa (AAA)	40	30	80	75	100	100
9	Grand naine (AAA)	40	10	60	15	100	50
10	Vella kappa (AAA)	0	0	0	0	0	0
11	Njali (AB)	0	0	10	3.5	35	19
12	Devan Kadali (AB)	66	20	66	25	100	25
13	Kunnan (AB)	33	16.66	66	41.7	100	66.67
14	Padatti (AAB)	0	0	0	0	0	0
15	Mysore Ethan (AAB)	0	0	0	0	0	0
16	Palayankodan (AAB)	5	3.7	10	9.7	60	47.5
17	Poovan (AAB)	55	13	80	15	100	75

18	Kunnur ethan (AAB)	0	0	0	0	0	0
19	Vannan (AAB)	0	0	25	16.7	67	25
20	Peyan (AAB)	0	0	0	0	0	0
21	Krishnavazha (AAB)	20	10	40	20	60	40
22	Perumpaadli (AAB)	0	0	0	0	0	0
23	Mottappoovan (AAB)	0	0	0	0	0	0
24	Chamba monthan (ABB)	5	3.1	100	75	100	83.3
25	Malamonthan (A BB)	0	0	0	0	0	0
26	Vella palayanthodan (ABB)	0	0	0	0	33	7.5
27	Malavazha	0	0	0	0	0	0

4.8.2 Virus Elimination through Meristem Culture

The meristem portion of BBrMV infected sucker was excised and inoculated to MS medium with BA for shoot induction. Shoots were developed within three weeks. Each one of the shoot thus developed was subcultured in fresh medium for the development of root and shoot. Four to five subculturing of each flask was made at 10-12 days interval. Within 45-60 days the development of root was occurred (Plate 14).

After proper establishment the plantlets with shoot and root were planted in small disposable cups for hardening and development (Plate 14). In the initial stages of hardening proper moisture was given. They were then transferred to large pots and kept under insect proof condition for four months under observation for the development of symptom.

Tissue culture plants thus developed from meristems of infected suckers were indexed for the presence of BBrMV using DAC-ELISA based on

Plate 14. Different growth stages of banana plant developed from the meristem of BBrMV infected sucker

which the meristem cultured plants were identified as BBrMV free plants (Table 27 and Plate 15).

Table 27 Reaction of meristem cultured plants developed from BBrMV infected suckers in DAC-ELISA

Sample	Absorbance at 405
Healthy sample	0.30
Meristem cultured plants	0.33
Infected sample	1.87

For healthy as well as meristem cultured plants the absorbance was almost equal (*i.e.*, about 0.30) but for the infected sample the absorbance was about six times higher *i.e.*, 1.87. Therefore DAC-ELISA confirmed the virus free nature of Nendran plants developed through meristem culture techniques from BBrMV infected suckers.

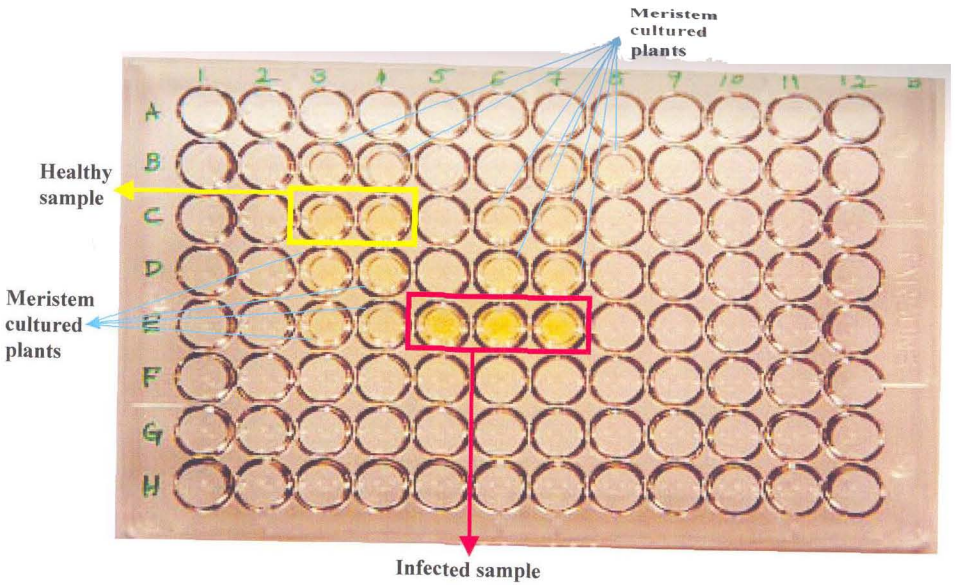


Plate 15. Reaction of banana plants developed from meristem of BBrMV infected sucker in DAC-ELISA

DISCUSSION

5. DISCUSSION

Banana (*Musa* sp.) is a vegetatively propagated cash crop grown as a staple food throughout tropical and subtropical areas of the world. In India, banana bract mosaic disease (Kokkan) was first reported from Kerala by Samraj *et al.* (1966). Later it was reported from Philippines by Magnaye (1979). The disease caused by BBrMV occur in plants of all age groups and is primarily transmitted through infected suckers. The wide spread incidence of the disease in Kerala is causing heavy reduction in yield.

Detailed studies on BBrMV have not been conducted in India so far. Hence the present investigation was undertaken with a view to throw light on the transmission of the virus, pathophysiological changes as a result of infection by BBrMV, purification of BBrMV, electron microscopy, production of antiserum for the pre-symptomatic detection of the virus, immunological studies and management of the disease by virus elimination through meristem culture.

5.1 SURVEY

Survey was carried out in the banana growing tracts of Thiruvananthapuram district to study the prevalence of the disease.

Survey of banana growing plots of four taluks of Thiruvananthapuram districts revealed that eventhough the disease was widely present in the district there was no significant difference between the taluks in the case of disease incidence (DI) and for per cent disease index (PDI) there was significant difference between taluks as well as panchayaths.

In the present investigation 0 – 100 per cent variation in DI and PDI was observed between panchayaths within taluks. Both DI and PDI were found to be increasing with progress of time. The first observation showed that the DI and PDI among different panchayaths varied between 0 – 30.98 and 1 – 4.81 respectively (Fig. 1 and Fig. 2). Observation made from same panchayaths after six months showed that the range of DI and PDI have increased to 17.79 – 39.67 and 3.29 – 6.91 respectively (Fig. 3 and Fig. 4).

Selvarajan (1997) conducted a survey for BBrMV in Nendran and Robusta after a gap of two years to study the reduction or increase in the spread of disease. The per cent disease index in Robusta and Nendran was 38.50 and 34.50 per cent respectively and it was nearly two to three fold as compared to the previous survey.

Survey conducted in Trichi revealed that the DI of BBrMD on Nendran was 67.13 and the PDI was 2.22. According to the survey there was reduction in height and girth of the plant and reduction in size and weight of the bunch (Thangavelu and Singh, 1996).

Sundararaju *et al.* (1999) observed BBrMV infection in different areas of Tamil Nadu. According to them the disease was noticed in Poovan, Nendran, Neypoovan, Robusta and Rasthali and the PDI varied from 0.33 to 60.10. At Malliampathi Nendran Orchard, in the early growth stages of Nendran BBrMV incidence was 44.00 per cent. After bunch emergence it was increased to 58.10 and at the time of bunch maturity it was 60.00 per cent.

None of the commonly cultivated varieties were found to be resistant to the disease. While conducting a survey in Philippines, Espino *et al.* (1990) found that most of the local cultivars were seen affected by BBrMD and the disease was common in Saba and Cardaba clones (Musa BBB). According to them the disease was also common in Cavendish varieties (Musa AAA).

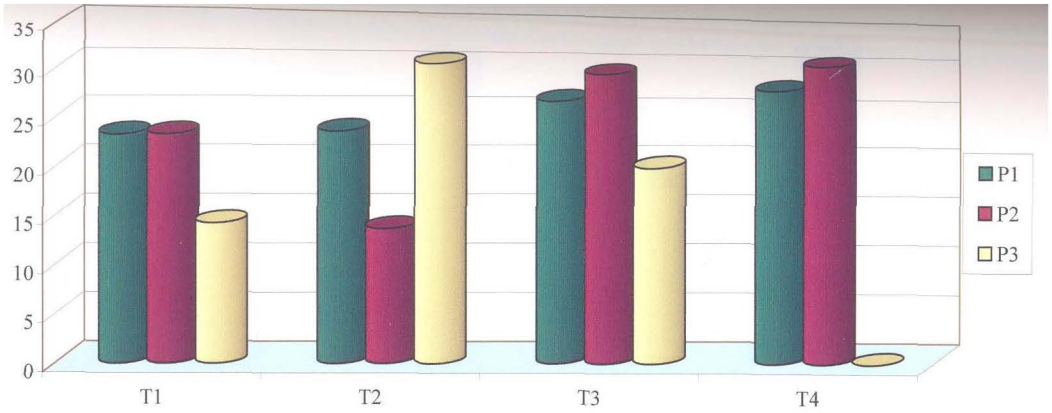


Fig. 1 Disease incidence of various panchayaths (three each from a taluk) showing prevalence of BBrMV in Thiruvananthapuram district (I observation)

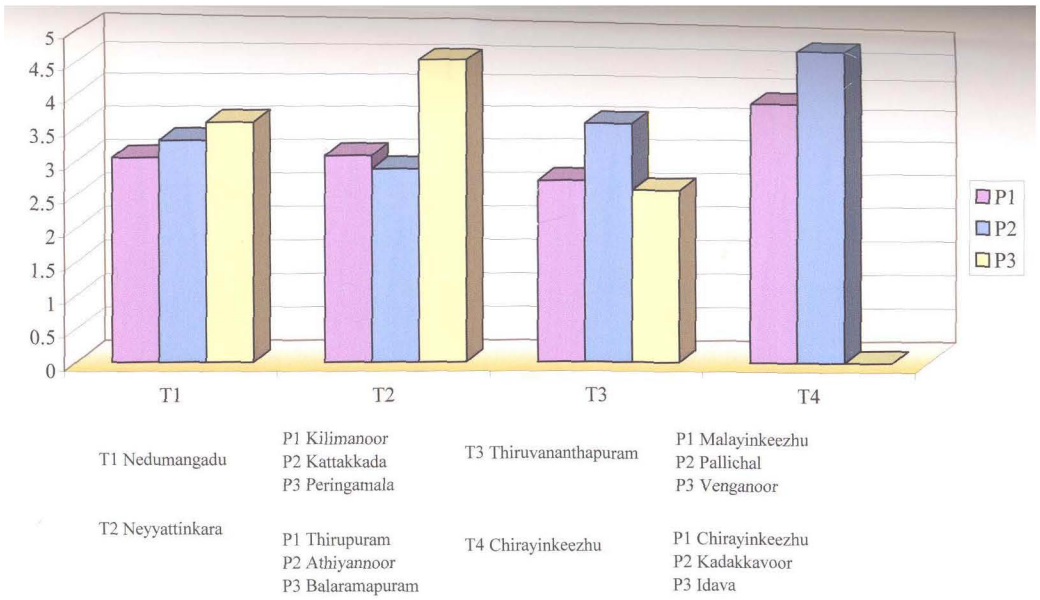
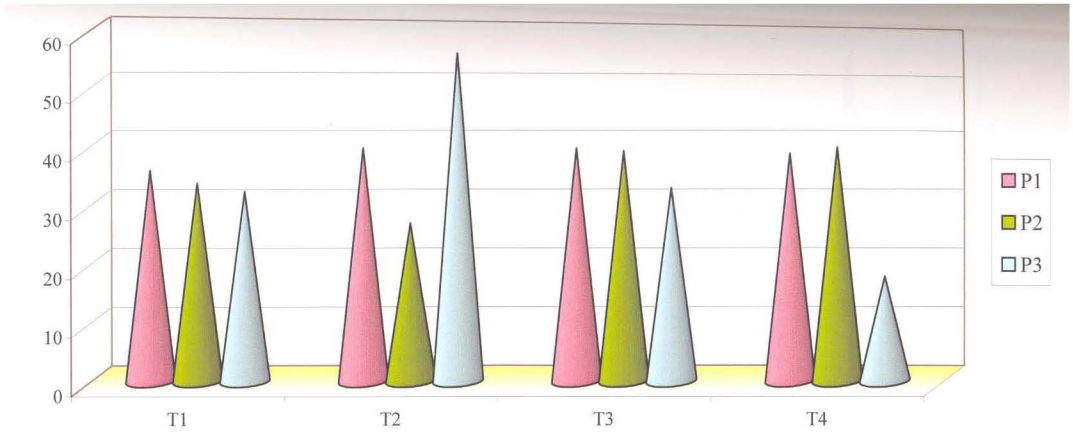


Fig. 2 Per cent disease index of various panchayaths (three each from a taluk) showing prevalence of BBrMV in Thiruvananthapuram district (I observation)



T1 Nedumangadu	P1 Kilimanoor P2 Kattakkada P3 Peringamala	T3 Thiruvananthapuram	P1 Malayinkeezhu P2 Pallichal P3 Venganoor
T2 Neyyattinkara	P1 Thirupuram P2 Athiyannoor P3 Balaramapuram	T4 Chirayinkeezhu	P1 Chirayinkeezhu P2 Kadakkavoor P3 Idava

Fig. 3 Disease incidence of various panchayaths (three each from a taluk) showing prevalence of BBrMV in Thiruvananthapuram district (II observation)

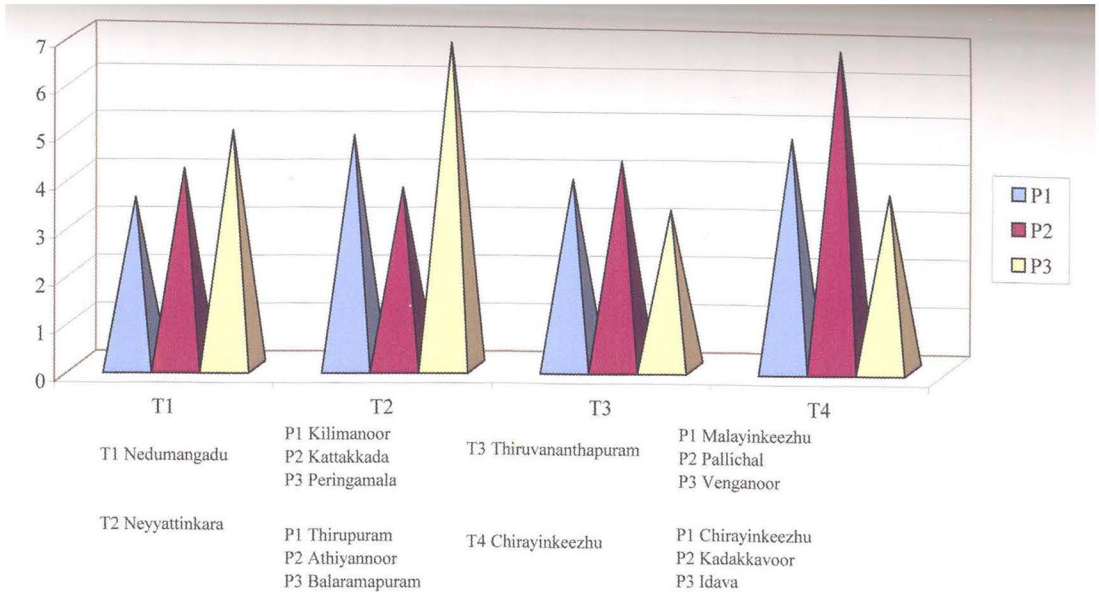


Fig. 4 Per cent disease index of various panchayaths (three each from a taluk) showing prevalence of BBrMV in Thiruvananthapuram district (II observation)

Observation made by Thomas and Magnaye (1996) was also in agreement with the present findings. They observed a widespread incidence of BBrMD in Cardaba (ABB/BBB), Saba (BBB) and Abutilon (BB) clones. Thangavelu and Singh (1996) conducted a survey in Trichi and reported the incidence of BBrMV in Robusta, Poovan, Neypoovan and Nendran. According to them BBrMV incidence varied from 0.22 to 8.80 per cent in Nendran.

Studies on symptomatology are essential for the early detection of the disease incidence. BBrMD affected plants showed chlorosis of leaves, longitudinal irregular reddish streaks on the pseudostem, necrotic streaks on the leaf axis, travellers' palm like appearance in some plants, mosaic like patches on the male inflorescence and production of small unmarketable malformed bunches and fingers. Symptomatological studies were in agreement with previous reports (Samraj *et al.*, 1966; Magnaye and Espino, 1990; Ravi *et al.*, 1992; Balakrishnan *et al.*, 1996; Thomas and Magnaye, 1996; Rodoni *et al.*, 1997; Smitha, 2001).

5.2 TRANSMISSION

Transmission studies conducted using infected planting materials indicated that infected suckers were the primary source of inoculum for BBrMV. This observation is in confirmity with those reported by many workers (Thomas and Magnaye, 1996; Pushkaran *et al.*, 1994; Bateson and Dale, 1995; Rodoni *et al.*, 1997; Frison *et al.*, 1998). Transmission studies conducted through the BBTV infected planting material by Estelitta (1998) revealed that infected suckers were the primary source of inoculum for BBTV. Allen (1978) and Jose (1981) also reported transmission of BBTV through infected suckers.

Mechanical transmission conducted using banana and other plants like *Nicotiana tabacum* and *Nicotiana benthamiana* as hosts with different buffers at different pH revealed that BBrMV was not mechanically transmissible. It is in confirmity with the earlier findings of Thomas and

Magnaye (1996). The failure to infect banana by crude sap from virus infected plant could be attributed to considerable amounts of latex and phenolic compounds present in banana which might interfere with virus infectivity and thereby prevent the transmission of virus from these plants to test plants (Yarwood, 1953; Hollings, 1957; Dale, 1987 and Wu and Su, 1990). Darnell (1923) tried to transmit BBTV by direct transmission of juice from bunchy top infected plants and was found to be unsuccessful. Observation made by Rajagopalan (1980) was in agreement with this. Estelitta (1998) conducted mechanical transmission of BBTV to banana and to other hosts with different buffers at different pH and found that BBTV was not mechanically transmissible. This supports earlier findings of Ross (1964) and Matthews (1982). Sap transmission studies attempted to transmit BBrMV to local lesion hosts like *Nicotiana tabacum* and *Nicotiana benthamiana* was also found to be unsuccessful. This finding was in agreement with Dickmann and Putter (1996).

Transmission of BBrMV using other inoculation methods *viz.*, pin-prick, syringe and knife inoculation were also found to be unsuccessful. Similar attempt was made by Rajagopalan (1980) for the transmission of BBTV and the result was negative. Darnell (1923) reported that use of hypodermic syringe injections and needles were unsuccessful for BBTV. Thomas and Magnaye (1996) reported that the spread of BBrMV through knives was unlikely.

In the present study, core grafting and root grafting were also attempted for the transmission of BBrMV and the result was found to be negative. Banana is a monocot plant and the graft unions fail to establish. Sastry *et al.* (1978) reported the successful transmission of banana bunchy top virus by core grafting in laboratory tests. Rajagoplan (1980) observed that such an experiment was unsuccessful for the transmission of BBTV. The negative result of the present study might be due to the failure of graft union of healthy and BBrMV infected suckers. According to Darnell

(1923), contact between roots of BBTV infected and healthy plants and grafting for disease transmission were not successful.

Insect transmission studies conducted with different aphid species showed that the aphid, *P. nigronervosa* alone could transmit the virus effectively (40 per cent transmission) whereas other vectors *viz.*, *A. gossypii* and *A. craccivora* failed to establish in banana plants and therefore could not succeed in acquisition and transmission of BBrMV.

The vector *P. nigronervosa* has been reported to transmit BBrMV (Bateson and Dale, 1995 and Castillo and Martinez, 1961). According to Thomas and Magnaye (1996) in addition to *P. nigronervosa, A. gossypii* and *Rhopalosiphum maidis* were also involved in the transmission of BBrMV. The same observation was also made by Sundararaju *et al.* (1999).

However in the present study *A. gossypii* failed to transmit the disease. In Kerala, the insect failed to establish on banana and that might be the reason for negative transmission.

Efficiency of transmission of BBrMV by *P. nigronervosa* was found to be increased with the increase in pre-acquisition fasting period of the vector. Maximum efficiency was noted when the insects were starved for a period of one hour (Fig. 5). Selvarajan and Padmanabhan (1997) starved the aphid *P. nigronervosa* for an hour and allowed to feed on infected banana for 5-10 minutes. On inoculation these aphids successfully transmitted BBrMV to healthy banana plants. Bateson and Dale (1995) reported the transmission of BBrMV by aphids in a non persistent manner in Philippines. Watson and Roberts (1939) reported that non-persistent relationship of the virus with its vector demands the pre-acquisition fasting. Since BBrMV is a non persistent virus in its aphid vector, fasting of the vector could increase the efficiency of transmission.

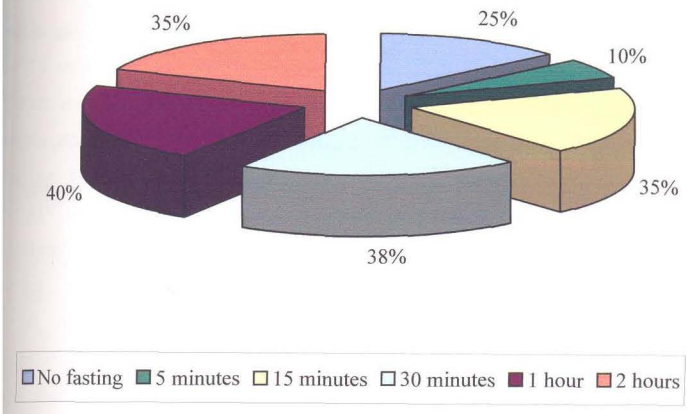


Fig. 5 Effect of pre-acquisition fasting of *P. nigronervosa* on the efficiency of transmission of BBrMV

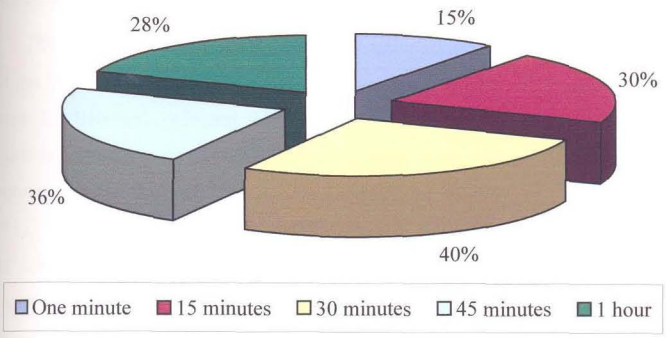


Fig. 6 Acquisition threshold of *P. nigronervosa* on the transmission of BBrMV

A short acquisition feeding period of one minute was sufficient for the aphid to become viruliferous. The optimum acquisition feeding period which gave the maximum per cent of infection (40 per cent) was found to be 30 minutes (Fig. 6). Mustaffa *et al.* (2001) reported 70 per cent transmission of BBrMV on banana by the vector *P. nigronervosa* after giving an acquisition feeding period of five minutes and inoculation feeding period of 30 minutes. Eventhough there is transmission of BBrMV after an acquisition period of one minute, in the present study the efficient transmission was observed with 30 minutes acquisition feeding period. Selvarajan (2003a) reported 70 per cent transmission of BBrMV by *P. nigronervosa* with acquisition feeding period of 5 min. and inoculation feeding periods of 30 min. The variation noted in the study with that of Mustaffa *et al.* (2001) and Selvarajan (2003b) might be due to the effect of environmental condition on symptom expression, or due to the difference in vector efficiency.

Estelitta (1998) reported an acquisition access period of two hours for transmission of BBTV by *P. nigronervosa.* Stover (1972) also reported that the efficiency of transmission of BBTV by *P. nigronervosa* increased with an increase in the acquisition time.

The present investigation on soil transmission of BBrMV revealed that the virus could not be transmitted through the soil collected from the basins of BBrMV infected plants. This indicates that no fungal or nematode vectors were involved in the transmission. This finding was in agreement with that of Thomas and Magnaye (1996) and Jose *et al.* (1971). Similar observation of BBTV transmission also through soil had been observed by various workers (Magee, 1940; Rajagopalan, 1980; Lockart, 1986).

5.3 PATHOPHYSIOLOGY

Physiological changes in banana infected with BBrMV was studied and changes in the content of total carbohydrate, chlorophyll, total phenol,

OD-phenol, protein and activity of defence related enzymes such as peroxidase (PO), Poly phenol oxidase (PPO) and Phenylalanine ammonia lyase (PAL) were studied at four growth stages of the banana.

In the present study the total carbohydrate content was found to be significantly decreased in BBrMV infected banana leaves at 3 MAP, 6 MAP and flag leaf stages whereas the content of same was more in infected bract. Decrease in total carbohydrate content has been reported in pigeon pea sterility mosaic virus (PSMV) infected pigeon leaves (Narayanasamy and Ramakrishnan, 1966). Reduction in carbohydrate content in host plants in response to pathogen attack has been reported by Goodman *et al.* (1967). According to them reduction was due to retarded photosynthesis and rapid utilization of starch and non-reducing sugars as substrates for increased respiration.

Reduction in the level of carbohydrate content in virus infected plants has also been reported by Narayanasamy and Ramakrishnan (1966). According to them this was to provide the substrate for accelerated respiration. These findings explains the reason for reduced sugar content in BBrMV infected plants as well as the increased carbohydrate content in infected bract.

On the other hand, an increased sugar content due to virus infection in various plants were also reported by many workers (Ramiah, 1978; Thind *et al.*, 1996; Srivastava and Tiwari, 1998). Increased sugar content in bhindi plant inoculated with yellow vein mosaic virus was reported by Bhagat and Yadav (1997). According to them accumulation of the sugar was by the disruption of normal phloem transport. Estelitta *et al.* (1993) found that the BBrMV infection did not result in the destruction of phloem of banana plants whereas more number of mechanical tissues were seen in diseased plants. According to them a good number of starch granules found in BBrMV infected flower primordia and inflorescence axis might

be the reason for increased carbohydrate content in BBrMV bract. Results obtained by Smitha (2001) was also in agreement with the present finding.

In the present study the contents of total chlorophyll, chlorophyll *a* and chlorophyll *b* were found to be significantly reduced in BBrMV infected plants. This result was in agreement with that of Smitha (2001). The reduction in total chlorophyll, chlorophyll *a* and chlorophyll *b* content due to virus infection was reported by several workers in other crops (Leal and Lastra, 1984; Sarma *et al.*, 1995; Dantre *et al.*, 1996; Thind *et al.*, 1996). Ramasamy (1967) also noticed a considerable reduction in the levels of total chlorophyll, chlorophyll *a* and chlorophyll *b* contents in the leaves of bunchy top affected banana plants. The reduction in chlorophyll on pathogen attack might be due to the diversion of plastid protein into virus (Bawden, 1954) or due to normal cell enzymes that attack chlorophyll (Goodman *et al.*, 1967). Ramiah (1978) and Ahmed *et al.* (1986) found that the reduction in chlorophyll content might be due to increased chlorophyllase activity of virus infected plants. Singh and Singh (1985) found that the loss in yield of cowpea due to cowpea mosaic virus infection was mainly attributed to the reduction in the rate of photosynthesis.

The content of total phenols and OD-phenols have been found to be correlated with disease resistance. The total phenol content was higher in BBrMV infected plants at six MAP and flag leaf stage compared to healthy plants. This result was in agreement with that of Smitha (2001). But at 3 MAP and bract stage healthy samples showed higher phenol content.

Increase in total phenols in infected plants were reported by many workers (Srivastava and Tiwari, 1998; Sarma *et al.*, 1995; Thind *et al.*, 1996; Banerjee and Kalloo, 1998). Jeeva (2001) reported that sweet potato feathery mottle virus infection on sweet potato contributed to an increase in the activity of hexose monophosphate (HMP) shunt pathway

which produced intermediates required for the synthesis of phenolic compounds. Farkas *et al.* (1960) found that the activation of phenol oxidizing enzyme was less in hosts due to systemic virus infection. Observation made by Rajagopalan (1980) was also in agreement with this. He opined that enzyme terminal oxidase utilizes phenolics as its substrate and accumulation of phenolics in BBTV affected banana might be due to the reduced activity of enzyme. In the present study activity of phenol oxidizing enzyme was found to be higher. Therefore increase in phenol content at certain stages of BBrMV infection might be due to increase in HMP shunt pathway as a result of BBrMV infection. The reason for lower phenol content of BBrMV infected plants at 3 MAP and bract stage might be due to the increased activity of terminal oxidases like PPO and PO.

The OD-phenol content was significantly higher in BBrMV infected banana plants at all stages of analysis and the content was maximum in infected bract. Kaur *et al.* (1998) reported higher content of OD-phenol and flavanols as a result of infection by cotton leaf curl virus when compared to susceptible varieties. Rajagopalan (1980) reported that OD-phenol content of bunchy top infected banana was higher than that of healthy banana. The increase in OD-phenol content in BBrMV infected sample might be the response of banana plant to virus infection.

The protein content of BBrMV infected plants was found to be significantly higher at all stages of analyses when compared to healthy.

Increased protein content due to virus infection was reported by Sarma *et al.* (1995). Investigations on enhanced protein content in virus infected plants were also reported (Singh and Singh, 1984; Singh and Singh, 1987; Yadav, 1988; Mayoral *et al.* 1989; Patil and Sayyad, 1991; Banerjee and Kalloo, 1998; Radhika, 1999 and Mali *et al.*, 2000). Estelitta (1998) reported that the increase in protein content of BBTV infected samples might be due to an increase in virus concentration as well as the production of other non viral proteins during active virus synthesis.

Nair and Wilson (1970) reported that the leaves of bunchy top virus infected banana plants contain higher percentage of total sugars, amino acids and tannin along with the presence of aspartic acid, alanine, methionine and isoleucine. The present finding was also in agreement with these. According to Uritani (1971) the increased protein content in virus infected plant might be due to the production of new PR proteins. Sindhu (2001) reported that inoculation of cowpea plants with black eye cowpea mosaic virus (BlCMV) caused a significant increase in total soluble protein content. The increased protein content in BBrMV infected plant might be due to the presence of coat protein of BBrMV as well as by the production of new PR proteins. Decreased protein content due to virus infection was also reported (Singh and Suhag, 1982; Thind *et al.*, 1996; Sarma *et al.*, 1995). Uritani (1971) reported that the decreased protein content might be due to the degradation of protein in the host.

Defence related enzymes were reported to act as an important factor in the induction of resistance (Dasgupta, 1988). The changes in the activities of PO, PPO and PAL were significantly higher in BBrMV infected banana plants in all stages of analysis with the exception that PAL activity at 6 MAP was on par with that of healthy banana. The PAL activity was highest in infected bract. This finding was in agreement with that of Smitha (2001). She found that PAL activity was higher in BBrMV plants.

Goodwin and Mercer (1972) found that the enzyme PAL served as the precursor for the synthesis of phenol. Since the BBrMV infected plant contain more amount of phenol the increased level of PAL might be the response of banana plant to BBrMV infection. Similar results were obtained by Zaid *et al.* (1992) in carnation etched ring virus and found that the substantial increase in the level of PAL activity along with the increase in phenolic content in response to infection show good correlation and suggest that virus infection has altered the activity of

enzymes of phenyl propanoid pathway and hence lead to accumulation of phenolics. Sindhu (2001) reported an enhanced PAL activity in BlCMV inoculated cowpea plants compared to healthy.

A significantly higher amount of PO and PPO was reported by Smitha (2001). The increased peroxidase activity was due to enhanced respiration (Pantanelli, 1912). Farkas *et al.* (1960) reported an increased PPO content in infected plants with necrotic lesion. Since the production of necrotic streaks was the common symptom of BBrMV infected plants, the increase in PPO might have resulted in necrosis of infected tissues. Increase in peroxidase activity in cowpea varieties susceptible to BlCMV was reported by Khatri and Chenulu (1970) and Sindhu (2001).

Ahmed *et al.* (1992) suggested that higher amount of phenols and their oxidation products like quinones formed by increased PO and PPO might be responsible for reduced virus multiplication and finally could lead to resistant reaction in yellow vein mosaic virus infected okra. Rajagopalan (1980) observed an increasing trend in peroxidase activity in BBTV infected plants. Gomathi *et al.* (1993) reported the increased activity of PO, PPO and PAL enzyme activity in banana infected with banana streak mosaic virus or banana common mosaic virus.

5.4 ELECTROPHORETIC ANALYSIS OF PROTEINS

SDS-PAGE analysis of BBrMV revealed that the virus infected plant sample had three major protein bands with estimated size of 31, 32 and 39 kDa and these extra bands revealed the presence of virus induced protein.

This result was in agreement with that of Thomas *et al.* (1997). According to them BBrMV possessed three major protein bands with relative molecular mass of 31, 37 and 39 kDa. These bands visible in coomassie stained SDS-polyacrylamide gels of dissociated BBrMV were absent from corresponding healthy preparations. Based on analogy with other potyviruses they concluded that these bands were probably the forms

of BBrMV coat protein and the heterogenity in size may have arisen from differential cleavage of the polypeptide precursor of coat protein. Bateson and Dale (1995) also reported a single major protein of 38 kDA in BBrMV infected plant samples. Estelitta (1998) conducted SDS-PAGE of different parts of BBTV infected banana and observed more number of protein bands in diseased parts than in healthy samples.

Based on the findings the present study confirmed the presence of BBrMV coat protein in BBrMV infected samples.

In India, this is the first successful attempt for the purification of BBrMV and protein determination.

5.5 ELECTROPHORETIC ANALYSIS OF ISOZYME

The difference in isozyme pattern could be useful in the detection of virus in the symptomless BBrMV infected plants. The present work was undertaken to study the enzyme alterations in healthy as well as BBrMD infected banana leaves.

Native PAGE carried out for PPO and PO showed that there was significant difference between healthy as well as BBrMV infected Nendran plants in isozyme production (both in number of bands as well as intensity of production) and it could be expressed as zymogram. Perusal of the available literature showed that earlier workers did not investigate the variation in isozyme production in virus infected as well as healthy banana.

Such studies due to virus infection was reported by many workers in other crops. The zymogram pattern of pintobean infected with southern bean mosaic virus exhibit two new peroxidase isozymes in infected plants (Farkas and Stahmann, 1966). Sindhu (2001) found that the isozyme polyphenol oxidase was more prominent in cowpea resistant to black eye cowpea mosaic virus compared to varieties susceptible to the virus.

Zymogram pattern of present study also showed that the isozymes PO and PPO were more prominent in BBrMV infected plants compared to

healthy banana. A single definite band of PO (Rm 0.63) (Fig. 7) was seen only for BBrMV infected sample whereas for PPO both healthy as well as infected samples exhibited a single band of Rm 0.65 (Fig. 8). But the intensity of production of PPO was more in infected sample as indicated by the thickness of the band.

5.6 BIOASSAY OF ENDOGENOUS GROWTH REGULATORS

The quantity of growth regulators *i.e.*, auxin, gibberlic acid and cytokinin were estimated from BBrMV infected as well as healthy Nendran fruits. The results indicated that all the above three growth regulators were found to be less in BBrMV infected fruits compared to healthy fruits.

So far no such studies were conducted in banana infected by BBrMV. Ramasamy (1967) observed that bunchy top diseased banana plant contained lower level of auxin than the healthy. Reduction in the growth of tomato plants infected with tomato spotted wilt virus due to the decrease in auxin content was reported by Grieve (1993). Smith *et al.* (1968) reported lower endogenous levels of auxins in *Lycopersicum esculenta*, *Phaseolus vulgaris* infected with curly top virus rather than healthy plants. Lower auxin content in TMV infected plants were reported by many workers (Pavillard, 1952; Pavillard and Beauchamp, 1957; Beauchamp, 1958) and with potato leaf roll virus (Jahnel, 1937; Lucas, 1937; Soding and Funke (1941). According to them,the dwarfing in many viral diseases was due to the lower auxin content of the tissues.

Haibao *et al.* (1997) reported less gibberlic acid content in banana bunchy top affected plants than the healthy banana plants.

5.7 CHARACTERIZATION OF BBrMV

Purification of BBrMV was done as per the procedure of Khurana *et al.* (1987) with slight modification. Banana contains considerable amount of latex and phenolics which interfere with virus extraction and purification

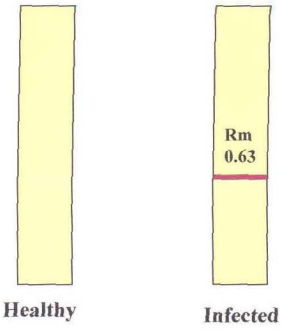


Fig. 7 Zymogram pattern for peroxidase isozyme induction in banana due to BBrMV infection

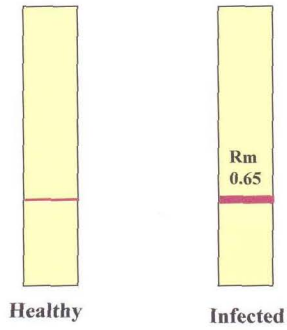


Fig. 8 Zymogram pattern for polyphenol oxidase isozyme induction in healthy as well as BBrMV infected banana

(Dale, 1987). Pulverization of frozen banana tissues under deep freeze condition before extraction greatly reduced such interference and appeared to be one of the main factors contributing to the successful extraction and purification of BBrMV. Purification of BBrMV from infected banana plants was carried out by number of workers (Espino *et al.*, 1990; Thomas *et al.*, 1997; Caruana and Galzi, 1997). Khurana *et al.* (1987) successfully purified potato virus.

Electron microscopic studies of the extract of BBrMV infected bract by leaf dip method showed flexuous rod shaped particles of about 725 x 12 nm size. This finding is in agreement with that of Rodoni *et al.* (1997); Khiem *et al.* (2000); Singh *et al.* (1996); Bateson and Dale (1995); Thomas *et al.* (1997) nd Caruana and Galzi (1997).

Antiserum was successfully produced against BBrMV with the partially purified virus preparation. BBrMV specific antiserum has been successfully produced by Thomas *et al.* (1997). Antisera against BBTV was successfully produced by Thomas (1984) and Estelitta (1998).

In the present studies the titre of the antiserum was found to be between 1:1024 and 1:512. Perusal of literature did not reveal such studies on BBrMV. Estelitta (1998) produced an antiserum against BBTV and found that it had a titre of 1:6512. Dubey *et al.* (1974) got an antiserum titre of 1:2048 for snakegourd mosaic. Rajagopalan (1980) reported the titre of the antiserum produced against BBTV was very less *i.e.*, 1:2 and this might be due to the low concentration of the virus in the plant.

In the present study detection of BBrMV in crude sap of infected banana by chloroplast agglutination test was found to be successful. No such study has been conducted to detect BBrMV from infected banana by earlier workers. However, Estelitta (1998) found chloroplast agglutination test as a successful tool for detection of BBTV.

The results of the microprecipitin test on slides showed that the antiserum produced was specific to BBrMV. No such test was conducted to detect BBrMV by earlier workers. Reghunadhan (1989) found that on micro precipitin test, snakegourd mosaic virus antigen produced dense precipitate with the antiserum produced against that. The results of the Ouchterlony's agar double diffusion test have confirmed the findings of the microprecipitin test on slides.

In double diffusion test definite precipitin band was formed between BBrMV infected sample and the developed antiserum whereas that was absent for healthy and buffer sample. This observation confirmed the presence of antibodies specific to BBrMV in the developed antiserum in sufficient concentration. Khurana *et al.* (1987) working with potato virus Y reported the development of sharp continuous bands for potato virus Y infected sample against its antiserum, and for healthy extracts this was absent.

In the present study double diffusion test was found to be efficient in early detection of BBrMV since it gave more pronounced visual observation compared to the other two methods. Basu and Giri (1993) made an observation similar to this. According to them double diffusion test was 2.5 times more sensitive than other precipitation test. Work done by Estelitta (1998) on banana infected with BBTV was in agreement with this.

ELISA is one of the very sensitive tests for detection, identification and characterization of virus. In the present study DAC-ELISA was performed with various samples of BBrMV infected banana plants using the developed antiserum. The test could detect the presence of BBrMV in crude extract of BBrMV infected young leaf, pseudostem and bract. The antiserum developed gave good result at dilution of 1:1024. In DAC-ELISA preformed absorbance values at 405 nm were higher in the bract of BBrMV infected plants (1.16) followed by pseudostem (0.62) and leaf

(0.62) when compared with healthy extracts. These observations clearly established that BBrMV was more concentrated on bracts, thus implying that the bract could be the best part for conducting ELISA test (Fig. 9). This result was in agreement with Thomas *et al.* (1997). They have got an absorbance value of 1.16 for BBrMV infected samples. This was in confirmity with the findings of Singh *et al.* (1996) where they reported that the concentration of BBrMV was found to be higher in infected bracts as compared to leaf shealth. Many workers successfully detected BBrMV in infected banana by ELISA (Singh *et al.*, 1996; Reddy *et al.*, 1996; Thomas *et al.*, 1997; Geering *et al.*, 1997; Sundararaju *et al.*, 1999).

In the present study DIBA was successfully used for the detection of BBrMV in infected banana plants. For infected samples definite purple coloured dots were developed on nitrocellulose membrane, where as these dots were absent for healthy extracts. Study conducted by Selvarajan (1997) was in agreement with this. Several studies on detection of viral diseases by DIBA has been conducted by many scientists. Moyer (1986) developed DIBA for the detection of sweet potato feathery mottle virus in sweet potato. Abad and Moyer (1992) performed membrane immunobinding assay for the detection of sweet potato feathery mottle virus (SPFMV). Selvarajan (2003b) reported that DIBA was more precise technique to detect BBrMV in infected samples compared to DAC-ELISA.

DIBA was successfully used for the detection of banana bunchy top virus by Geering and Thomas (1996). Since BBrMV infected banana plants could be symptomless in early stages of infection and there was 100 per cent transmission through infected planting material as well as for indexing of banana suckers, these tests were important.

5.8 MANAGEMENT OF THE DISEASE

Screening of banana germplasm collection at College of Agriculture, Vellayani and Banana Research Station, Kannara was carried out for resistance against BBrMV based on symptom expression under field

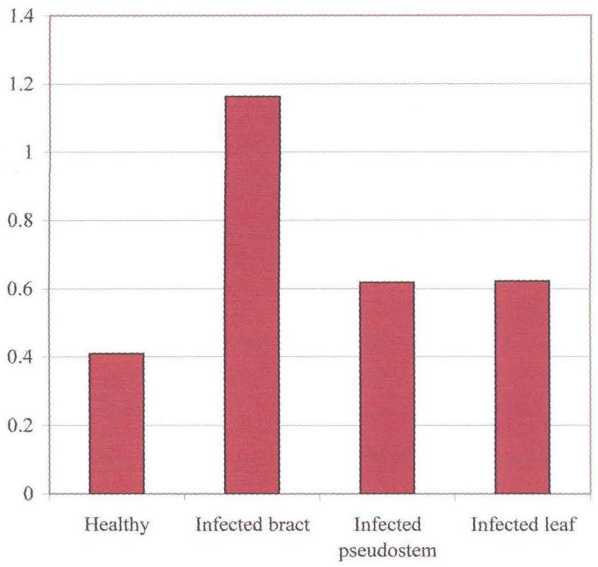


Fig. 9 Reaction of different parts of banana plants infected by BBrMV in Direct Antigen Coating ELISA

conditions and accessions without any visible symptom were identified. Among the 180 accessions screened in the present work at Banana Research Station, Kannara, 162 accessions were found to be free from any of the symptoms of BBrMD. This observation did not mean that these 162 accessions were resistant to BBrMV. Observation made at germplasm collection at Instructional Farm, Vellayani showed that most of the cultivated varieties of banana *i.e.,* Robusta, Chenkadali, Vellakappa, Palaynkodan and Njalipoovan, found symptomless at Kannara were found to be affected by BBrMV. The systematic phytosanitation and replanting of disease free suckers might be the reason for comparatively less incidence of the disease at Kannara.

Another important observation noted in the present study was that in Kannara the accessions with 'A' genome compared to those with 'B' genome were resistant to BBrMV. This observation was in agreement with that of Estelitta *et al.* (1993). According to them among 152 varieties screened for disease, 34 showed BBrMV symptom. They also reported that varieties having 'B' genome were found to be more susceptible to the disease. The comparative resistance to accessions with 'A' genome might be due to the hardy nature of those banana plants.

Sundararaju *et al.* (1999) screened germplasms of Kannara and found that the varieties Nendran, Kunnan, Wather, Bodles Altafort, Pachanadan, Manjeri Nendran, Thenkadali, Co-1, Rasthali, Karpuravalli, Dudhasagar, KNR 2/95, Kullan, Paloor, Chandra Bak, Dakshin Sagar, Vannan and Lacatan were susceptible to BBrMV infection. Based on the survey conducted at Peringamala they recorded BBrMV infection on Palayankodan, Red banana, Monthan, Rasthali, Jawa, Krishnavazha, Kareem Kadali, Sakkai, Matti, Mysore ethan, Padathi, Pisanglinin and Adukkon. According to them in Vellayani more than 50 per cent Neypoovan was affected by BBrMV.

In the present study it was also noted that in some of the banana clumps, suckers had the symptom, but not in mother plants. It might be due to the transmission of BBrMV to suckers by aphids from neighbouring infected plant. Another possibility was the expression of symptoms in later stages (*i.e.*, after a latent period). Observation made by Sundararaju *et al.* (1999) on Nendran orchard at Malliampathi was in agreement with this finding.

Meristem culture was attempted in the present study to find out its possibility in eliminating the virus from planting materials. The result revealed that about 100 per cent of meristem cultured plants were free of BBrMV (Fig. 10). MS medium with Benzyl adenine (5 mg/l) was used for the induction of tissue cultured banana plants. The plants were ready for hardening with in 70-80 days. After hardening the plants were observed under *in vivo* condition for four months and confirmed the absence of virus by visual observation as well as DAC-ELISA.

Singh and Verma (2001) indexed BBrMV incidence by ELISA in tissue culture banana plantation in farmers' field and found that there the incidence of BBrMV was low *i.e.*, 4.1 per cent. Rames and Zamore (1999) successfully used meristem culture combined with heat treatment of infected suckers and produced about 63.2 – 100 per cent BBrMV free plants. According to Thomas and Dietzgin (1991) tissue culture could be used to eliminate BBTV from the germplasm. Several workers successfully produced banana plants free of viral diseases by meristem tip culture (Shinchaun and Hongii, 1998; Frison *et al.*, 1998). According to Caruana (1997) the only way of controlling virus disease of banana was by the use of virus free planting materials. Dhingra *et al.* (1988) reported that combination of meristem tip culture and thermo therapy at 37°C was very effective in eradicating potato virus X (PVX) in potato tubers.

Observations in the survey conducted at Edava panchayath as well as studies on the screening trial of germplasm collection at Kannara showed

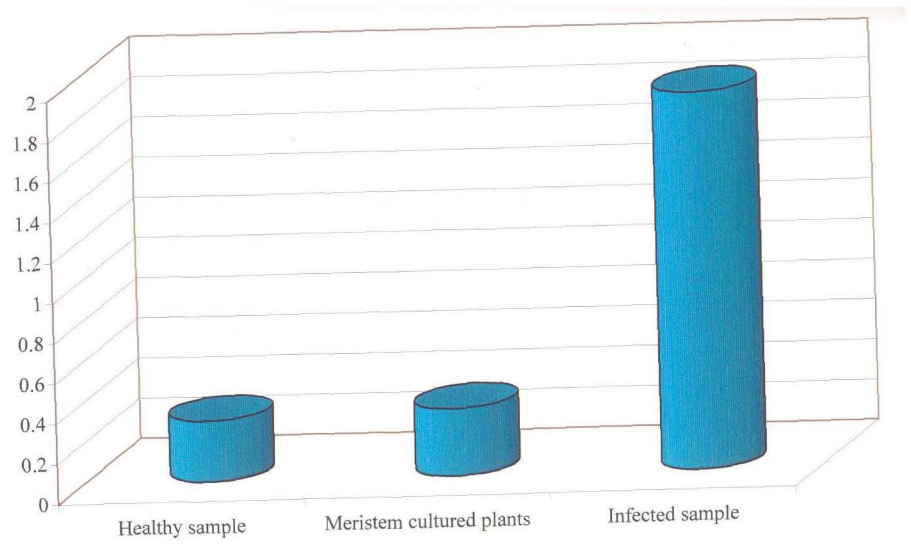


Fig. 10 Reaction of banana plants meristem cultured from BBrMV infected suckers in DAC-ELISA

that when healthy planting material free from BBrMD were used, the incidence of the disease in the field was very low. Transmission studies also showed that there was 100 per cent transmission through infected suckers whereas 40 per cent transmission obtained with the vector *P. nigronervosa*. Hence the study vividly highlighted the importance of using meristem cultured plants as planting material as well as proper indexing of suckers using sensitive test such as ELISA coupled with stringent phytosanitation and vector control will help in the effective management of this widespread malady.

SUMMARY

6. SUMMARY

Survey conducted in four taluks of Thiruvananthapuram district revealed that banana bract mosaic virus (BBrMV) is widely prevalent in the district. Eventhough there was no significant variation between taluks, the different panchayaths among taluks showed significant variation in disease incidence. In the case of per cent disease index, both the taluks and panchayats showed significant variation. There was increase in disease incidence with increase in the age of the plantation.

Banana bract mosaic disease (BBrMD) incidence resulted in mosaic symptoms on the surface of flower bracts of affected plants. Other symptoms include unusual longitudinal reddish streaks on outer surface of pseudostem and it was pronounced on young plants and also on suckers. As the plant grows, these streaks turned brownish to black necrotic regions and also appeared on petiole and peduncle.

In some of the infected plants the orientation of the leaves became fan shaped (traveller's palm appearance)

The BBrMV was transmitted mainly through infected suckers (100 per cent transmission) and sap transmission was not found to be successful.

Different types of grafting *viz.*, core grafting and root grafting could not be established properly between healthy as well as BBrMV infected plants, therefore failed to transmit the disease. The result of soil transmission of the disease was found to be negative, hence revealed that no soil-borne organism was involved in the transmission of the disease.

Insect transmission studies conducted with different aphids *i.e.*, *Pentalonia nigronervosa*, *Aphis gossypii* and *A. craccivora* showed that *P. nigronervosa* was the efficient vector of BBrMV (40 per cent

transmission) whereas the other two aphids failed to establish on banana. Pre-acquisition fasting period of one hour and acquisition threshold of 30 min. was found to be optimum for the efficient transmission of BBrMV by *P. nigronervosa*.

Total carbohydrate and phenol content of banana plants infected by BBrMV was less in different stages of analysis of infected plant (*i.e.*, three months after planting, 6 months after planting and flag leaf stage) compared to healthy whereas it was *vice-versa* at bract stage. Coming to total chlorophyll, chlorophyll *a* and chlorophyll *b* the content of these were less in BBrMV infected banana plants compared to healthy. The content of orthodihydroxy phenol (OD-phenol), protein and the activities of defence related enzymes such as peroxidase (PO), polyphenol oxidase (PPO) and phenylalanine ammonialyase (PAL) were found to be enhanced in BBrMV infected plants.

Electrophoretic analysis of the protein of BBrMV infected sample by Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) revealed the presence of viral protein (that of BBrMV) as three extra major protein bands with estimated size of 31, 32 and 39 KDa.

Electrophoretic analysis of the isozyme revealed that the induction of isoenzymes (*i.e.*, PO and PPO) by BBrMV infection as bands in infected samples compared to healthy. Intensity of production was also more in infected samples and it was evident by thickness of the band.

Status of endogenous growth substances was estimated and found that the content of all three growth regulators *i.e.*, auxin, cytokinin and gibberlic acid were low in BBrMV infected samples compared to healthy.

Purification of BBrMV was done successfully from BBrMV infected banana plants and polyclonal antiserum against that was produced by injecting Newzealand White female rabbit. Direct antigen coating enzyme linked immunosorbent assay DAC-ELISA was conducted and the

presence of antibodies in the antiserum was confirmed. The antiserum developed against BBrMV gave an optical density of 1.6 at 405 nm at 1:1024 titre of antibody.

Electron microscopy of the BBrMV infected sample by leaf dip method showed long flexuous rods measuring approximately 725 x 12 nm.

For the detection of BBrMV several serological tests were conducted. Chloroplast agglutination test was attempted and successfully detected BBrMV from crude sap of infected banana. Micro precipitin test was also found to be useful for the detection of BBrMV. Agar double diffusion test was conducted and detected the presence of BBrMV in infected sap. ELISA and Dot immuno binding assay (DIBA) was successfully carried out for the detection of BBrMV from infected tissues containing even lower concentration of BBrMV.

Screening of germplasm accessions at Banana Research Station, Kannara revealed that out of 180 accessions screened only 27 accessions showed characteristic visual symptoms of BBrMD. It was observed that varieties with B genome were found to be more susceptible to the disease compared to those with A genome. Observation made from germplasm collection at Vellayani showed that the commonly cultivated varieties which were found to be symptomless at Kannara were also found to be susceptible to BBrMV. This might be due to the selection of BBrMV free suckers at Kannara for planting. So none of the varieties could be categorized as resistant type to BBrMV.

Meristem culture was attempted to produce BBrMV free plants from infected suckers. Using this technique about 100 per cent BBrMV free plants were produced. These plants were maintained for four months under insect proof condition and the virus free nature of the plants were confirmed periodically by conducting DAC-ELISA.

Present study on BBrMV revealed that there was 100 per cent transmission through infected planting material. Data from the survey as well as screening of germplasm collection at Banana Research Station, Kannara and Instructional Farm, Vellayani showed that use of disease free planting material and management of vector were the only choice to manage the disease. Selection of disease free planting material on the basis of visual observation is quite difficult and the indexing of suckers were cost effective. Therefore, the use of tissue cultured banana plants developed through meristem culture technique help to manage the disease to a great extent.

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7. REFERENCES

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*Original not seen

APPENDICES

APPENDIX - I

Buffers used in sap transmission

1. 0.1 M Sodium borate buffer (pH 8.0)

A. Boric acid (0.2 M) 1.237 g / 100 ml
B. Borax $Na_2B_4O_7$-$10H_2O$ 1.907 g/100 ml

3 ml of A mixed with 7 ml of B, diluted to a total of 20 ml

2. Potassium phosphate buffer

A. 0.1 M Potassium dihydrogen phosphate 6.084 g/ 500 ml
B. 0.1 M Dipotassium hydrogen phosphate 8.079 g/ 500 ml

0.1 M (pH 7.2) – 28 ml of A mixed with 72 ml of B
0.05 M (pH 7.2)– 28 ml of A mixed with 72 ml of B, diluted to a total of 200 ml

(0.01 M DIECA) – 0.225 g / 100 ml

3. 0.1 M Sodium phosphate buffer (pH 7.2)

A. 0.1 M Sodium phosphate monobasic anhydrous 5.999 g / 500 ml
B. 0.1 M Sodium phosphate dibasic dihydrate 8.899 g / 500 ml

28 ml of A mixed with 72 ml of B

4. 0.1 M Citrate buffer (pH 6.2)

A. 0.1 M Citric acid 2.101 g / 100 ml
B. 0.1 M Sodium citrate 2.940 g / 100 ml

1.6 ml of A mixed with 18.4 ml of B

5. 0.1 M Tris buffer (pH 7.2)

Tris 24.23 g / 1000 ml

22.5 ml of 0.2 N HCl mixed with 25 ml of Tris , diluted to a total of 50 ml

APPENDIX – II

Arnons reagent

Arnons reagent was prepared by dissolving 10 g of sodium nitrate ($NaNO_2$) and 10 g of sodium molybdate (Na_2MOO_4) in 100 ml of distilled water. The reagent was stored in a coloured bottle in cool dark place.

APPENDIX – III

Preparation of stock dye solution for estimation of protein

100 mg of Coomassie brilliant blue G-250 was dissolved in 50 ml of 95 per cent ethanol and 100 ml of concentrated orthophosphoric acid was added. The volume was made upto 200 ml with water and kept at 4°C. The working dye was prepared just before use by diluting the stock solution to five times with water.

APPENDIX – IV

0.1 M sodium phosphate buffer - pH 6.5

A. 0.2 M solution of mono basic sodium phosphate = 27.8 g / 1000 ml
B. 0.2 M solution of di basic sodium phosphate = 53.65 g NA_2HPO_4 .7 H_2O in 1000 ml
68.5 ml of A mixed with 31.5 ml of B diluted to a total of 200 ml.

APPENDIX – V

0.1 M borate buffer

A. 0.2 M boric acid – 12.4 g / 1000 ml
B. 0.05 M solution – 19.05 g / 1000 ml
50 ml of A mixed with 30 ml of B diluted to a total of 200 ml

APPENDIX – VI

Protein denaturing solution

10 M urea – 80 ml
1 M NaH_2PO_4. $2H_2O$ (pH 8) - 5 ml
1 M Tris (pH 8) – 1 ml
5 M sodium chloride – 2 ml
Makeup volume to 100 ml by adding 12 ml of distilled water.

APPENDIX – VII

SDS-PAGE staining and destaining solution

Staining solution

Coomassie brilliant blue R 250 - 0.1 g
Methanol – 40 ml
Acetic acid – 10 ml
Distilled water – 50 ml

Destaining solution

As above without Coomassie brilliant blue.

APPENDIX VIII

Stock solutions for isozyme analysis

1. Tris-glycine electrode buffer stock solution, pH 8.3

Tris	6.00 g
Glycine	28.8 g
Distilled water	1000 ml

2. Electrode buffer

Dilute the Tris-glycine electrode buffer stock solution with distilled water in a 1:9 ratio.

3. Tris-chloride buffer stock solution, pH 8.9

HCl, 1N	48.00 ml
Tris	36.6 g
TEMED	0.23 ml
Distilled water	100.00 ml

4. Tris-chloride buffer stock solution, pH 6.7

HCl, 1N	48.00 ml
Tris	5.98 g
TEMED	0.46 ml
Distilled water	100.00 ml

5. Resolving gel acrylamide stock solution

Acrylamide	28.00 g
N 'N'-methylene bisacrylamide	0.74 g

Make up to 100 ml with distilled water. Store in a dark bottle at 4°C for upto 2 weeks.

6. Ammonium persulphate solution

Ammonium persulphate	0.1 g

Dissolve in 1 ml distilled water. Make fresh each time.

7. Bromophenol blue solution

Bromophenol	25 mg

Make up to 10 ml with Tris-chloride buffer solution, pH 6.7

8. Resolving gel solution (for one 1.5 mm gel, 7.5 %)

Tris-chloride buffer Solution, pH 8.9	5 ml
Resolving gel acrylamide solution	10 ml
Distilled water	25 ml
Ammonium persulphate solution	300 µl

9. Staking gel solution (for one 1.5 mm gel, 4 %)

Tris-chloride buffer stock Solution, pH 6.7	2.5 ml
Resolving gel acrylamide solution	3.1 ml
Distilled water	14.1 ml
Ammonium persulphate	300 µl

APPENDIX IX

IAA stock solution (10^{-3})

Stock solution of 10^{-3} M IAA was prepared by dissolving 17.5 mg of IAA in little amount of alcohol and the volume was made upto 100 ml using the phosphate buffer having pH 5.9. Different concentrations of IAA ranging between 10^{-4} and 10^{-9} M were prepared by serially diluting the stock solution.

APPENDIX X

Cytokinin (Benzyl adenine) stock solution

Stock solution of 10^{-5} M was prepared by dissolving 22.525 mg Benzyl adenine (BA) in little amount of 1 N sodium hydroxide and the volume was made up to 100 ml using 0.2 mM phosphate buffer (pH 5.9). Different concentrations of BA ranging between 10^{-5} to 10^{-8} M were prepared by serially diluting the stock solution.

APPENDIX XI

Dinitro Salicylic Acid (DNS) Reagent

DNS reagent was prepared by dissolving one gram dinitro salicylic acid, 200 mg crystalline phenol and 50 mg sodium sulphite in 100 ml one per cent NaOH solution. It was stored at 4°C.

Appendix XII

0.01 M phosphate buffer (pH 7.0)

A. 0.02 M solution of monobasic sodium phosphate – 2.78 g / 1000 ml
B. 0.02 M solution of dibasic sodium phosphate – 5.37 g Na_2HP_O4. $7H_2O$/1000 ml

39 ml of A mixed with 61 ml of B diluted to a total of 200 ml.

0.1 M Phosphate buffer pH 7.5

A. 0.2 M solution monobasic sodium phosphate 27.8 g in 1000 ml
B. 0.2 M solution of dibasic sodium phosphate – 53.65 g Na_2HPO_4. 7 H_2O / 1000 ml

Appendix XIII

Electron microscopy

0.1 M Potassium phosphate buffer pH 7.5

A. 0.1 M potassium dihydrogen phosphate 6.08 g in 500 ml
B. 0.1 M dipotassium hydrogen phosphate 8.08 g in 500 ml

16 ml of A mixed with 84 ml of B

Appendix XIV

Buffers for DAC- ELISA

1. Phosphate buffered saline (PBS - pH 7.4)

Sodium chloride	-	8.0 g
Potassium dihydrogen phosphate	-	0.2 g
Disodium hydrogen phosphate	-	1.1 g
Potassium chloride	-	0.2 g
Sodium azide	-	0.2 g
Water	-	1000 ml

0.5 ml Tween 20 (0.05%)

2. Coating buffer (pH 9.6)

Sodium carbonate	-	1.59 g
Sodium bicarbonate	-	2.93 g
Sodium azide	-	0.2 g
Water	-	1000 ml

3 Substrate solution (pH 9.8)

Diethanolamine	-	97 ml
Sodium azide	-	0.2 g
Water	-	800 ml

Add HCl to give pH 9.8

Appendix XV

Amido black stain for agar gel double diffusion test

1. Staining solution

a) Amido black 10 D stain 0.6 g
b) Methanol 45 ml
c) Glacial acetic acid 10 ml
d) Distilled water 45 ml

2. Destaining solution

Same as above without amido black stain

APPENDIX XVI

Stock solutions for DOT-Immunobinding Assay (DIBA)

1. Stock solution buffer (Tris-buffer saline, TBS, pH 7.5)

0.02 M Tris	4.84 g
0.5 M NaCl	58.48 g

Adjust the pH to 7.5 with 1 N HCl and make up to 2 litre. This is used as Wash solution.

2. Antigen extraction buffer (TBS – 5o0 mM DIECA)

Add 11.25 g diethyl dithiocarbonate (DIECA) to 1 litre TBS

3. Blocking solution (TBS-SDM)

Add 5.0 g spray dried milk (SDM) to 100 ml TBS

4. Antibody and enzyme-conjugate diluent/buffer

Same as TBS-SDM

5. Substrate buffer (pH 9.5)

0.1 M Tris	12.11 g
0.1 M NaCl	5.85 g
5 mM $MgCl_2.6H_2O$	1.01 g

Adjust the pH to 9.5 with 1 N HCl and make upto 1 litre

6. Substrate solution

Solution A

Nitro Blue tetrazolium (NBT)	75 mg
DMFA	1 ml

Solution B

Bromo Chloro Indolyl Phosphate (BCIP)	50 mg
DMFA	1 ml

Store solutions A and B refrigerated in amber bottles. Add NBT to 0.33 mg/ml and BCIP to 0.175 mg/ml to the substrate buffer just before use.

7. Fixing solution (pH 7.5)

10 mM Tris	1.21 g
1 mM EDTA	0.29 g

Adjust the pH to 7.5 with 1 N HCl and make upto 1 litre

All buffers contain 0.02 % sodium azide as a preservative.

APPENDIX XVII

Stock solutions for MS basal medium

Sl. No.	Constituents	Amount of chemical taken (mg)	Amount mg l^{-1}	Solution ml l^{-1}
1.	Stock solution I (250 ml 10 X)			
	$MgSO_4$ 7 H_2O	3700	370	25 ml
	KH_2PO_4	1700	170	
	KNO_3	19000	1900	
	NH_4NO_3	16500	1650	
2.	Stock solution II (100 ml 20 X)			
	$CaCl_2$ 2 H_2O	8800	440	5 ml
3.	Stock solution III (100 ml – 100 X)			
	H_3BO_3	620	6.2	1 ml
	$MnSO_4$. 4 H_2O	1690	16.9	
	$ZnSO_4$. 2 H_2O	860	8.6	
	$Na_2M_0O_4$. 2 H_2O	25	0.25	
	KI	83	0.83	
4.	Stock solution IV (100 ml – 20 X)			
	$FeSO_4$ $_{7H}2O$	556	27.8	5 ml
	Na_2 EDTA. 2 H_2O	746	37.3	
5.	Stock solution V (250 ml – 500 X)			
	Cu SO_4. 5 H_2O	12.5	0.025	.5 ml
	CO Cl_2. 6 H_2O	12.5	0.025	
6.	Stock solution VI (100 ml-100X)			
	Thiamine HCl	10	0.1	1 ml
	Pyridoxin HCl	50	0.5	
	Nicotinic acid	50	0.5	
	Glycine	200	2	
	Myoinositol – 100 mg			
	Sucrose – 30 g			
	Agar – 7.5 g			

* Dissolve $FeSO_4$. 7 H_2O and Na_2 EDTA 2 H_2O separately in 45 ml distilled water by heating and constant stirring. Mix the two solutions adjust the pH to 5.5 and add distilled water to makeup the final volume to 100 ml.

All the prescribed stock solutions, chemicals and hormones were taken in a beaker and the volume was made up to 1 litre. The agar was dissolved in the medium by melting before autoclaving.

PURIFICATION AND IMMUNODETECTION OF BANANA BRACT MOSAIC VIRUS

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Abstract of the
thesis submitted in partial fulfilment of the requirement
for the degree of

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ABSTRACT

The study entitled "Purification and immunodetection of banana bract mosaic virus" was conducted in College of Agriculture, Vellayani, Thiruvananthapuram during 2000-2003.

Survey conducted in Thiruvananthapuram district revealed that banana bract mosaic virus (BBrMV) is widely prevalent in different taluks of the district. Zero to 100 per cent variation was observed for disease incidence and per cent disease index was significantly different between taluks. The disease was found to increase with progress of time. Studies on varietal reaction revealed that none of the commonly cultivated varieties were resistant to BBrMV. Symptomatological studies showed that the characteristics symptoms of banana bract mosaic disease were longitudinal irregular reddish streaks on pseudostem, chlorosis of leaves, necrotic streaks on petiole and bract, travellers' palm appearance, severe reduction in bunch size and formation of malformed fingers. Mechanical transmission of BBrMV through different means, graft transmission and soil transmission were unsuccessful. The aphid *Pentalonia nigronervosa* Coq. was identified as efficient vector of BBrMV (40 per cent transmission) with pre-acquisition fasting of one hour and acquisition threshold of 30 minutes.

Carbohydrate content was less in BBrMV infected plants compared to healthy at all stages of analysis except at bract stage. The phenol content was more in infected banana plants at six months after planting and flag leaf stage whereas it was higher in healthy plants at three months after planting and at bract stage. Content of OD-phenol, protein, activity of peroxidase, polyphenol oxidase and phenylalanine ammonialyase were found to be more in banana bract mosaic virus infected plants.

Electrophoretic analysis of BBrMV infected samples through SDS-PAGE revealed the presence of three extra bands (of virus) with molecular

weight of 31, 32 and 39 kDa. Electrophoretic analysis of isozymes through native gel revealed the production of peroxidase isozyme in infected plants and the over expression of polyphenol oxidase isozyme in plants infected with BBrMV. Bioassay of endogenous growth regulators showed that the content of auxin, cytokinin and gibberlic acid was less in BBrMV infected fruits compared to healthy.

The virus, BBrMV was purified from infected young leaf and the antiserum was developed in New Zealand white rabbit by giving intramuscular injection of partially purified virus. Titre of antiserum was tested using DAC-ELISA and it was determined as 1 : 1024. Electron microscopic studies of infected plant sample revealed that the virus particles were long flexuous rods with an average size of 725 x 12 nm. Detection of BBrMV infected plant parts was done using various immunological techniques like chloroplast agglutination, microprecipitin, Ouchterlony's agar gel double diffusion test, DAC-ELISA and dot immuno binding assay and all were found to be efficient for the detection of BBrMV.

Germplasm collection at Banana Research Station, Kannara were screened for banana bract mosaic disease resistance and found that varieties with 'A' genome were found to be more susceptible to the disease compared to those with 'B' genome. Screening of varieties at Instructional Farm, Vellayani showed that all of the commonly cultivated varieties were susceptible to BBrMV. For the production of virus free planting material meristem culture technique was attempted using meristems of BBrMV infected suckers. Virus free nature of the developed plants was confirmed through DAC-ELISA and it was found that about 100 per cent plants developed through meristem culture were free of BBrMV. Based on the survey and screening of germplasm collection, it was concluded that strict phytosanitation and use of virus free planting materials, preferably meristem cultured plants will help to manage the disease to a great extent.