# SYMPTOMATOLOGY AND MOLECULAR DIAGNOSIS OF BANANA STREAK VIRUS DISEASE

By

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## THESIS

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# CERTIFICATE

Certified that this thesis, entitled "**Symptomatology and molecular diagnosis of Banana streak virus disease** " is a record of research work done independently by **Ms. Divya.C.R** under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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# DECLARATION

I, Divya.C.R.(2007-11-106) hereby declare that this thesis entitled "**Symptomatology and molecular diagnosis of Banana streak virus disease**" is a bonafide record of research work done by me during the course of research and this thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title of any other University or Society.

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We, the undersigned members of the advisory committee of **Ms. Divya.C.R,** a candidate for the degree of **Master of Science in Agriculture**, with major field in **Plant Pathology**, agree that the thesis entitled **"Symptomatology and molecular diagnosis of Banana streak virus disease"** may be submitted by her in partial fulfilment of the requirement for the degree.

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Divya C.R

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# *Introduction*

# 1. INTRODUCTION

The banana (*Musa* spp.) is a crop of global importance in terms of income security to millions of small farmers throughout the developing countries. It is the world's fourth most important commodity after rice, wheat and corn and is produced in tropical and subtropical regions of developing countries. India is the largest producer of banana in the world, contributing 19.71 per cent to the global production with a total production of 19.19 million tonnes from an area of 0.565 million ha (Singh, 2008). Among the different banana growing states of India, though Kerala ranks third in area, the production and productivity is low. This is due to the polyclonal system of cultivation that too, mostly under homestead and perennial conditions. This provides a favourable environment for pests and diseases to sustain throughout the year, affecting the productivity. Banana is infected by several viral diseases and among which, Banana streak disease is an important one affecting banana production world wide. Banana streak virus infection in banana and plantains assumes significance as it affects plant growth, fruit yield and quality, besides causing hindrance to germplasm exchange, and certification of *in vitro* plantlets for international trade (Lockhart *et al.*, 2000).

Banana streak disease (BSD) was first described as 'La mosaique a tirets' occurring on the banana cultivar 'Poyo' in Nieky in Valley Cote d' Ivoire (Ivory Coast), Africa in 1974 (Lassoudiere, 1974). Banana streak badnavirus (BSV), the casual agent of banana streak disease was first identified from Morocco in 1985 (Lockhart, 1986). This disease is now known to affect many clones of *Musa*. In India, this disease was first confirmed on banana cv. Mysore (Thangavelu and Singh, 1996) and on other cultivars (Ram and Singh, 1999; Cherian *et al.*, 2002).

BSV detection based on symptom is often confusing because of similarity of symptoms with those of the Cucumber mosaic virus. The sporadic nature of the

symptom expression throughout the year makes the diagnosis difficult (Jones and Lockhart, 1993). The effect of temperature on symptom expression of banana streak infected plants was reported by Dahal *et al.* (1998). Dahal *et al.* (2000) reported that the symptom expression and severity were more pronounced during the rainy season than in dry weather. Symptom expression may be totally lacking in plants derived from *in vitro* multiplication.

BSV infects only *Musa* and *Ensete* and is not transmissible by mechanical means. Kubiriba *et al.* (2001) reported the role of mealy bug as vector of BSV. Mealy bug transmission of BSV by *Planococcus citri* Risso has been demonstrated else where. But these mealy bugs have not been reported on banana in India and their role in BSV transmission is not fully investigated.

Many attempts have been made to develop sensitive, reliable methods for BSV indexing. These have been only partially successful in screening *Musa* for BSV, because these techniques failed to detect different isolates of the virus (Lockhart and Olszewski, 1993 and Lockhart, 1995). Dahal *et al.* (1998) reported that BSV antigens can be detected by triple antibody sandwich enzyme linked immuno sorbent assay (TAS-ELISA) and the concentration of BSV antigens was high in symptomatic tissue but very low in asymptomatic tissue. Polyclonal antibodies to BSV isolate was produced and used for the detection of serologically unrelated BSV isolates using double antibody sandwich enzyme immuno assay (DAS-ELISA) (Ndowora,1998).

PCR mediated amplification using two pairs of oligonucleotide primers based on badnavirus sequence was shown to be a potentially useful method for the detection of all BSV isolates (Lockhart and Olszewski, 1993). A PCR based diagnostic method was also developed for the genome of a Nigerian isolate of BSV and is sequenced and comprised of 7389 bp which was organized in a manner characteristic of badnaviruses (Harper and Hull, 1998). Geering *et al.* (2000) made phylogenetic analysis which

revealed that all the BSV isolates were more closely related to each other than to any other badna virus. The use of immunocapture polymerase chain reaction as a reliable and sensitive method which can detect episomal BSV was attempted by many workers (Wetzel *et al.*, 1992; Harper and Hull, 1998; Harper *et al.*, 1999 and Yang *et al.*, 2003).

A real time PCR was developed for the rapid detection of episomal banana streak virus using strain BSV-OL in banana which carries integrated BSV sequences by Delanoy *et al.* (2003). Cherian *et al.* (2004) cloned and sequenced a part of the genomes of two isolates of BSV from Kerala. However, the isolates from other varieties of Kerala have not been sequenced. Le Provost *et al.* (2006) developed a multiplex immunocapture polymerase chain reaction (M-IC-PCR) for the detection of BSV and *Musa* sequence tagged micro satellite site (STMS) primer were selected and used in combination with BSV species-specific primer in order to monitor possible contamination by *Musa* genome DNA. Selvarajan *et al.* (2008) standardized PCR based technique to detect integrants and BSV infection.

Recently in Kerala, banana streak disease is emerging as a great hindrance to the multiplication of quality planting material both by conventional and *in vitro* propagation approaches. Hence, it is essential to make a detailed study on the Banana streak disease on commercial varieties of banana grown in Kerala. The present investigation will give the details on the symptomatology, role of root mealy bug-*Geococcus* sp. in the transmission of BSV, sources of resistance and PCR based techniques for quick and reliable indexing of planting materials. The outcome of this study will facilitate distribution of healthy quality planting materials, both suckers and tissue culture plants to the farmers, thereby increasing the production and productivity of banana in the state.

# Review of literature

# 2. REVIEW OF LITERATURE

Banana is the most important fruit crop of Kerala. The cultivation of banana is often hindered by many biotic constraints such as diseases. Among viral diseases, banana streak virus (BSV) has emerged as a serious threat to banana cultivation in Kerala recently. The literature related to the studies done so far on the disease in India and abroad are reviewed in this chapter.

## 2.1 Occurrence

Banana streak disease (BSD) has recently aroused great concern to the worldwide banana and plantain cultivation. The disease was first described as 'La mosaique a tirets' occurring on the banana cultivar 'Poyo' in Cote d' Ivoire in 1974 (Lassoudiere, 1974). Later it has been reported from more than 40 countries of Africa, Asia, Europe, Oceania and South and Central America (Diekmann and Putter, 1996; Pasberg *et al.*, 1996). Numerous out breaks of the disease occurred among promising banana breeding lines and micro propagated inter-specific *Musa* hybrids (Dallot *et al.*, 2001; Cherian *et al.*, 2002; Lheureux *et al.*, 2003). The geographical distribution of this disease in the world is summarized in Table 1.

The occurrence of this disease in India on different banana cultivars has been reported by many of the workers. The incidence of BSV on banana cultivar Poovan (AAB) grown in Tamil Nadu was reported to be 11.60 to 100 per cent while the yield loss was up to 50 per cent and on cultivar Robusta (AAA) the yield loss was reported to be only 0.70 per cent (Thangavelu and Singh, 1996). In Maharashtra, 5-10 percent incidence of BSV was noticed on *in vitro* propagated plants of Cavendish banana (Ram and Singh, 1999). Now in India, the presence of BSV has been confirmed from other states of India viz., Karnataka, Andhra Pradesh, West Bengal, Bihar, Assam, and

Gujarat (Singh, 1996). The occurrence of the disease on Mysorepoovan (AAB) and hybrids BRS-1 (AAB), BRS-2 (AAB) was reported from Kerala by Cherian *et al.* (2002).

**Table 1.Geographical distribution of Banana streak disease**

| Region | Country/ location | References |
|---|---|---|
| Europe | Portugal (Madeira) | Jones and Lockhart,1993 |
| | Spain (Canary Islands) | Diekmann and Putter,1996 |
| Africa | Cote d' Ivoire | Yot-Dhauthy and Bove,1966 |
| | Uganda | Dabek and Waller,1990; Tushmereirwe *et al.*,1996. |
| | Morocco | Lockhart,1986 |
| | Rwanda | Sebasigari and Stover,1988 |
| | Tanzania (incl.Zanzibar) | Dabek and Waller,1990;Vuylsteke *et al.*,1996 |
| | Madagascar | Jones and Lockhart,1993 |
| | Mauritius | Jones and Lockhart,1993 |
| | Nigeria | Jones and Lockhart,1993 |
| | South Africa | Jones and Lockhart,1993 |
| | Benin | Diekmann and Putter,1996 |
| | Cape Verde | Diekmann and Putter,1996 |
| | Ghana | Diekmann and Putter,1996 |
| | Guinea | Diekmann and Putter,1996 |
| | Kenya | Diekmann and Putter,1996 |
| | Malawi | Vuylsteke *et al* .,1996 |
| | Cameroon | Gauhl and Pasberg-Gauhl, 1997; Gauhl *et al.* ,1999 |

| | | |
|---|---|---|
| Latin America | Brazil | Jones and Lockhart,1993 |
| Caribbean | Costa Rica | Brunt *et al.*,1987;Diekmann and Putter,1996 |
| | Cuba | Jones and Lockhart,1993 |
| | Ecuador | Jones and Lockhart,1993 |
| | Grenada | Jones and Lockhart,1993 |
| | Guadeloupe | Jones and Lockhart,1993 |
| | Honduras | Jones and Lockhart,1993 |
| | Jamaica | Jones and Lockhart,1993 |
| | Trinidad | Jones and Lockhart,1993, |
| | Colombia | Reichal *et al.*,1997 |
| | Haiti | Lockhart,1999 |
| USA | Florida,Virgin Islands | Diekmann and Putter,1996 |
| Asia-Pacific | China | Jones and Lockhart,1993 |
| | India | Singh, 1996; Thangavelu *et al.*, 1996 ; Cherian *et al.*, 2002. |
| | Indonesia | Diekmann and Putter,1996 |
| | Malaysia | Diekmann and Putter,1996 |
| | New Caledonia | Diekmann and Putter,1996 |
| | Papua New Guinea | Diekmann and Putter,1996 |
| | Philippines | Diekmann and Putter,1996 |
| | Sri Lanka | Diekmann and Putter,1996 |
| | Thailand | Diekmann and Putter,1996 |
| | Vietnam | Diekmann and Putter,1996 |
| | Taiwan | Su *et al.*,1997 |
| | Australia | Thomas *et al.*,1998 |

| | | |
|---|---|---|
| | Tonga | Thomas *et al.*,1998 |
| | Western Samoa | Thomas *et al.*,1998 |

## 2.2 Symptomatology

The naturally occurring isolates of BSV produced widely varying symptoms on *Musa* genotypes. Lockhart (1986) reported reduction in bunch size and malformation of fingers of banana variety, Dwarf Cavendish due to BSV infection. Frison and Putter (1989) reported that diseased plants were stunted and sometimes failed to flower. Even if they flowered, bunches were very small and poorly filled. The leaves had chlorotic streaks, which in some cases turned golden or necrotic. Foliar symptoms caused by BSV initially resembled those caused by CMV. But in later stages necrotic streaks occured only on the leaves affected by BSV. A characteristic of BSV infection is the sporadic nature of symptom expression. The infected plants might not express streak symptoms on leaves for several months. The sporadic nature of the symptom expression throughout the year made the diagnosis based on foliar symptoms very difficult (Jones and Lockhart, 1993). BSV infection expressed a significant effect on bunch size and fruit quality especially when floral initiation and early bunch development coincided with the period of increased virus synthesis (Lockhart and Olszewski, 1993).

Gauhl and Gauhl (1995) described symptom of BSV as discrete whitish yellow flecks or short streaks on the leaf lamina, discrete longer yellow to orange colored streaks, having a dark brown to black necrotic centre followed by complete black streaks. Some times eye shaped yellow streaks form a mosaic pattern on the leaf lamina, streak symptoms on leaf petioles, bracts and on mature fruit. Severe mosaic like streaks resulted in distortion of the leaf lamina. Severe infection resulted in stunting of

plant and decay of the cigar leaf followed by plant death. Internal necrosis of the pseudostem and lengthwise cracking of the outer leaf sheaths were also observed. Bunches sometimes burst out from the side of the pseudostem.

According to Dahal *et al.* (1998) the symptoms of the disease appeared as discrete white to yellow flecks or spindle-shaped lesions on the leaf lamina, shorter pseudostem internodes, resulting in rosette like stunted leaves. Often the cigar leaf turned necrotic with distorted lamina. Incomplete emergence of bunch, distorted bunch or bunch bursting through the pseudostem, death of the plant before maturity was also reported. Daniells *et al.* (1998) reported the appearance of broad yellow lines on the leaf lamina parallel to the midrib were observed on banana variety 'Williams'(AAA,Cavendish Subgroup) from Australia. Singh (2002) described the symptoms as chlorotic flecking, lethal systemic necrosis, yellow brown and black streaking, cigar-leaf necrosis, basal pseudostem splitting and internal deformed bunches. Cherian *et al.* (2002) reported that symptoms of the disease include the appearance of chlorotic and necrotic brown streaks on the leaves.

## 2.3 Causal agent

BSV belongs to genus Badnavirus and family Caulimoviridae (Van Regenmortal, 2000). The virions are non-enveloped, bacilliform, 130-150nm x 30nm in size, and contain a circular double stranded DNA genome of size 7.5kb (Medberry *et al.*, 1990; Lockhart and Olszewski, 1993).

It has been shown that BSV genomic sequences are integrated into the genome of *Musa* and *Ensete* (Lafleur *et al.*, 1996). Meanwhile, Harper *et al.* (1999) and Ndowora *et al.* (1999) suggested that this integrated sequence could lead to episomal forms. The strain BSV-Onne is only one of the at least four strains of BSV, which showed great

genomic and serological heterogeneity and poorly characterized (Lockhart and Olszewski, 1993).

Strong evidence exists that integrated sequences that can be activated are associated with the B-genome of cultivated *Musa,* whereas *Musa* AAA group (cv.Williams) contains a non activable virus integrant (Geering *et al.,* 2001). Episomal particles were detected in two plantain cultivars and the hybrid CRBP-3 (Tey cheny *et al.,* 2004).

## 2.4 Host range

The natural hosts of badnaviruses are the plants with centre of origin and diversity in South East Asia and Australia (Lockhart and Olszewski, 1993). The badnaviruses have a very narrow host range. Crops infected with badnavirus include sugarcane, cocoa, pineapple, black pepper, citrus and yam.

Weed hosts do not play any role in the epidemiology of banana streak disease (Francki, *et al.,* 1979). BSV has been found naturally only in *Musa* and transmitted experimentally only to *Musa* and *Ensete.*

## 2.5 Transmission

BSV and other members of the genus badnavirus are transmitted by mealy bugs (Pseudococciidae) (Lockhart and Olszewski, 1993). Mealy bug vectors of badnaviruses include species of *Pseudococcus, Planococcus, Planococcoides, Ferrisia, Saccharicoccus* and *Dysmicoccus* (Brunt, 1970; Lockhart *et al.,* 2000). The virus is transmitted by *Planococcus citri* Risso and *Saccharicoccus sacchari* Cockerell, both of which colonize banana (Lockhart and Olszweski, 1994). Jones and Lockhart (1993) reported BSV to be transmitted in a semi-persistent manner by mealy bug (*Planococcus citri* Risso) from banana to banana under green house condition.

Sugarcane mealy bug (*Saccharicoccus sacchari* Cockerell) transmitted another badnavirus, sugarcane bacilliform virus (SCBV) from sugarcane to banana (Jones and Lockhart, 1993). Adult mealy bugs are sedentary, the early instars or crawlers are highly mobile. Young instars are more efficient than old instars. Mealy bugs transmit badnaviruses in a semi persistent manner (Lockhart and Olszeweski, 1993).

Su (1998) reported that BSV was transmitted by two mealy bug bio-types of *Planococcus citri* Risso, wild biotype with short trait, *Planococcus citri-ST* and a new biotype with long trait, *Planococcus citri-LT*. Kubiriba *et al.* (2001) reported that the acquisition feeding period for *Dysmicoccus brevipes* Cockerell was three days and the detection of BSV in the recipient banana plants was four weeks after transmission using pineapple mealy bugs and six weeks after inoculation using sugarcane mealy bugs. Singh (2002) reported that the acquisition feeding period for citrus mealy bug was one day and found that young instars were more efficient than old instars in transmitting the virus. The alate adults with degenerated mouth parts could not transmit the virus. Selvarajan *et al.* (2006) reported that BSV could be transmitted semi persistently through striped mealy bug, *Ferrisia virgata* Cockerell and not by sugarcane pink mealy bug and also the mealy bugs from guava and pineapple failed to transmit the virus. Recently, a new mealy bug *Geococcus* sp. is reported to cause wide spread infestation in the commercial banana orchards of Kerala (Smitha *et al.*, 2005).The role of this mealy bug as a vector of BSV is not yet investigated.

Several aphid species such as *Aphis gossypii Glover* and *Rhopalosiphum padi* L. and *Pentalonia nigronervosa* Coquerel which briefly colonize banana, failed to transmit the virus (Lockhart, 1986; Kubiriba *et al.*, 2001).

Daniells *et al.* (1995, 2001) reported seed transmission of BSV in banana cv. Mysore and is transmitted through infected suckers up to 100 per cent.

All attempts to transmit the virus by mechanical inoculation have failed, even when young *in vitro* plants were inoculated (Lockhart, 1986).

## 2.6 Screening of germplasm for the occurrence of BSV

Screening banana clones with *Musa balbisiana* genome for the presence of endogenous pararetrovirus (EPRV's) sequences of banana streak viruses (BSV) has become a pre-requisite in banana breeding programmes (Lheureux *et al.*, 2003).

The first reports of Banana streak disease were from cultivars in the Cavendish sub group (AAA) in Cote d'Ivoire (Lassoudiere, 1974) and Morocco (Lockhart, 1986). Sebsigiri and Stover (1988) reported that Banana streak disease was found in Kabungo, 'Ney Poovan' (AB), 'Pisang Awak' (ABB) and the East African high-land beer-making cultivars of the 'Lujugira-Mutika' subgroup (AAA). In the Bukoba district of Tanzania, the disease was seen on 'Gros Michel' (AAA) and cooking cultivars in the 'Lujugira-Mutika' (AAA) subgroup. They also reported the occurrence of the disease in Zanzibar on 'Giant Cavendish' (AAA, Cavendish subgroup) and 'Pukusa' (AAB, syn. 'Silk').

Later, Dabek and Waller (1990) recognized banana streak symptoms on 'Mjenga', (AA, syn. 'Sucrier'),'Kipaka' (AA, syn. 'Paka'), 'Bukoba'(AAA, syn. 'GrosMichel'), 'Paji'(AAA). 'Mzunga Mwekunda' (AAA, syn' Red'), Mzunga Mweupe (AAA, syn. 'GreenRed'), 'Wkono Wa Tembo' (AAB, 'Horn plantain type'), 'Kikonde Kenya' (AAB, syn. 'Mysore') and 'Kijakaze' (AAB, Pome Subgroup). These accessions were maintained in germplasm collection in Zanzibar.

Daniells *et al.* (1998) reported the occurrence of the disease in 'Williams' (AAA, Cavendish Subgroup) from Australia. Jones (2002) reported the association of BSV particles in cultivars like 'Kisubi'(AB, 'Ney Poovan') from Burundi, 'Pisang Berangan'(AAA, syn. 'Lakatan) in Malaysia, 'Pisang Raja' from India and Malaysia.

cultivars in the AAB Maia 'Maoli-Popoulu' sub group from Colombia and Australia and 'Monthan' (ABB, Bluggoe sub group) from Cote d' Ivoire. The virus was also seen in some AAAB hybrids from the breeding programme in Guadeloupe, Honduras, Nigeria and Brazil.

In India, this disease was first confirmed on banana cv. Poovan (Thangavelu and Singh, 1996) and on other cultivars by Ram and Singh, 1999 and Cherian *et al.*, 2002. Saraswathi *et al.* (2007) reported that among the 265 accessions from India screened for five BSV EPRV's using PCR-based markers, 32 accessions were free of all five EPRVs, whereas 48 accessions were free of BSV-Obino I'Ewai' (BSV-OI), BSV-Goldfinger (BSV-Gf) and BSV-Mysore (BSV-Mys) EPRVs. All accessions were free of BSV-Vietnam (BSV-AcVN).

## 2.7 DETECTION OF VIRUS

Detection of BSV is problematic due to serological and genomic heterogeneity of virus isolates (Lockhart and Olszewski, 1993), the erratic appearance of symptoms (Dahal *et al.*, 1998) and the uneven distribution of the virus in plants. Diagnosis based on foliar symptoms may be very unreliable because of the sporadic nature of symptom expression throughout the year (Jones and Lockhart, 1993). Symptoms may be totally absent, or may be indistinct, under certain conditions. Symptom expression was also lacking in plants derived from *in vitro* multiplication. The biotechnological tools like serological and Polymerize Chain Reaction (PCR) based molecular techniques are quick and reliable for the early detection of banana streak virus (Harper and Hull, 1998).

### 2.7.1 Electron Microscopy

Bacilliform particles measuring 119 × 27nm were detected by electron microscopy, in diseased banana plants (Lockhart, 1986). BSV isolates could be detected by immunosorbent electron microscopy (ISEM) using partially purified leaf-tissue extracts (Bouhida. *et al.*, 1993). Dahal *et al.* (1998) reported the presence of bacilliform virus particles measuring 30 × 130-150 nm in the partially purified preparations of BSV infected leaf material. Immunosorbent electron microscopy of viral minipreps, was standardized using multi-strain antiserum of BSV (Diekmann and Putter, 1996; Thottapilly *et al.*, 1998). The EM of infected leaves of banana showed typical bacilliform particles which indicated that the streak like symptoms were associated with the infection of Banana streak disease (Cherian *et al.*, 2004). These symptoms were earlier misunderstood for Cucumber mosaic virus infection.

### 2.7.2 Serology

BSV is serologically similar to Sugarcane bacilliform virus (SCBV) (Lockhart and Autray, 1988). Naturally occurring isolates of BSV were characterized by high degree of both serological and genomic variability, a factor which has created considerable difficulty in developing reliable antigen-based or genome-based detection methods (Lockhart and Olszewski, 1993). To overcome this problem, polyclonal rabbit, mouse and chicken antisera were raised against a mixture of serologically distinct BSV isolates (Ndowora, 1998). A more sensitive procedure known as the triple antibody sandwich (TAS-ELISA) was employed using rabbit IgG as secondary antibody for detecting the virus isolates (Ndowora and Lockhart, 1997). Dahal *et al.* (1998) reported that the concentration of BSV antigens could be detected by TAS-ELISA.

Thottapilly *et al.* (1998) produced polyclonal antibodies against Nigerian isolate of BSV with a titre between 1:10000 and 1:40000 and also different ELISA protocols

double antibody sandwich (DAS-ELISA), antigen coated plate (ACP- ELISA), direct antigen coated (DAC- ELISA) and triple antibody sandwich (TAS-ELISA) were compared for their reliability and sensitivity to detect BSV antigen in infected leaf extracts. To detect a wider range of BSV isolates in infected banana and plantain material TAS-EIA is more sensitive than DAS-EIA (double antibody sandwich enzyme immuno assay (Ndowora and Lockhart, 2000). The concentration of BSV antigens would be varied in different plant parts. The possible variation of the virus was assessed by ELISA using a broad spectrum antiserum (Harper *et al.*, 2002). Agindotan *et al.* (2003) reported the production of two monoclonal antibodies and these antibodies together with the polyclonal antibodies were used for the detection of BSV.

### 2.7.3 Genome based Techniques

Lafleur *et al.* (1996) standardized the technique of hybridization with a probe derived by PCR amplification using badnavirus specific degenerate oligonucleotide primers. Harper *et al.* (2002) standardized PCR based detection of BSV using degenerate primers set involving concentrating the virus in sap by polyethylene glycol precipitation followed by IC-PCR or DB-PCR(Directly bound PCR). Cherian *et al.* (2004) reported about the detection of BSV by PCR and also cloned and sequenced a part of the genome of BSV. Detection of Indian isolate of BBTV and BSV with duplex PCR and three viruses including BBrMV by multiplex PCR has been reported by Selvarajan (2008).

Wetzel *et al.* (1992) and Harper *et al.* (1999) developed an IC-PCR based strategy to detect episomal BSV in banana and plantain. IC-PCR combines, the immunological capture of BSV viral particles by a polyclonal antiserum (Ndowora, 1998) and amplification of the viral ORF III domain encoding the RNaseH and severe transcriptase. BSV-Onne is integrated into the *Musa* genome and special precaution such as immuno capture PCR was used to distinguish integrated forms from episomal

viral DNA (Harper *et al.*, 1999; Ndowora *et al.*, 1999). Geering *et al.* (2004) constructed and characterized three BAC libraries and found positive upon screening using M-IC-PCR (multiplex immunocapture-PCR) assay which was developed for the detection of episomal BSV. M-IC-PCR developed for the detection of BSV using *Musa* sequences tagged microsatellite site (STMS) primers was reported by Le-Provost *et al.* (2006).

# Materials and Methods

# 3. MATERIALS AND METHODS

The present study on the "Symptomatology and molecular diagnosis of Banana streak virus disease" was carried out in the Department of Plant Pathology, College of Horticulture, Vellanikkara and Banana Research Station, Kannara during 2008 to 2010. The experiment site is located at an elevation of about 58 MSL. The average temperature of the region is 28°C and the annual rainfall ranges between 2700 and 3000mm, distributed between two rainy seasons. Relative humidity varies between 77 and 94%.

The details of the materials used and the techniques adopted for the investigation are described below.

## 3.1 COLLECTION AND MAINTENANCE OF BSV CULTURE

The culture of banana streak virus (BSV) maintained at Banana Research Station, Kannara was used for the study. The naturally infected suckers were also collected from different fields of banana and planted in pots kept in insect proof net house.

Healthy tissue culture plants of banana variety Nendran (AAB) banana raised in pots were used for various studies.

## 3.2 SYMPTOMATOLOGY

A field trial was laid out by planting infected and healthy suckers of banana accessions viz., Chandrabale (AAB), Kalibale (AAB), Motta poovan (AAB), Mysore poovan (AAB) and Nendran (AAB) with five replications. The field was surrounded with fence made of nylon net.

### 3.2.1 Development of symptoms

The development of symptoms of the disease at each growth stage of the plants was documented. The symptoms expressed on different parts of the plant like leaves, petiole, pseudostem, male buds and bunches were recorded.

### 3.2.2 Varietal variation of symptoms

The expression of symptoms on five different banana accessions was recorded and the varietal variation in the development of symptoms was documented.

### 3.2.3 Impact of BSV infection on agronomic and yield characters

The agronomic parameters like height, girth and numbers of leaves of all the plants at six months after planting were recorded. The yield parameters like bunch weight, number of hands, number of fingers, fruit weight, length and girth of the finger of both healthy and infected plants were also recorded.

### 3.2.4. a Impact of environmental factors on symptom expression

The seasonal variability of symptom expression on naturally BSV infected plants was recorded. The expression of the symptom on each leaf was rated as per the scoring method reported by Dahal *et al.* (2000) with slight modifications. The expression of the symptom on each leaf of the infected plant was rated using 0-6 scale (0-without symptoms, 1= up to 1%, 2=1-5%, 3=6-15%, 4=16-33%, 5=34-50% and 6=51-100%). Based on this scoring, symptom severity index, percentage disease incidence, percentage disease severity and coefficient of infection were calculated.

Symptom Severity Index (SSI) = 0 (a) + 1 (b) + 2 (c) + 3 (d) + ..... 6 (f)/n

where a, b, c, ....f = Number of leaves with respective scores

1, 2, 3,.....n = Total number of leaves

Percentage Disease Severity (PDS) =

$$\frac{0(a) + 1(b) + 2(c) + \ldots. 6(f)}{\text{Total number of leaves}} \times \frac{100}{\text{Maximum grade}}$$

where a, b, c, ....f = Number of leaves with respective scores

Percentage Disease Incidence (PDI) = $\frac{\text{Number of infected plants}}{\text{Total number of plants}} \times 100$

Coefficient of Infection (CI) = $\frac{\text{PDS} \times \text{PDI}}{100}$

The biometric characters like height, girth and number of leaves of the infected and healthy plants were recorded. The yield parameters of both healthy and infected varieties were also recorded. The relationship of the symptom expression with weather parameters was worked out by statistical analysis of the data using correlation method.

## 3.3 SCREENING OF FIELD GENE BANK

The field gene bank comprising of 295 accessions maintained at BRS, Kannara were screened at monthly intervals from planting till harvest for the natural occurrence of the banana streak disease. Scoring of infected suckers was done based on 0-6 scale. Based on scoring data symptom severity index was calculated as mentioned in section 3.2.3.a.

## 3.4 TRANSMISSION STUDIES OF BSV

The transmission studies were carried out under insect proof net house.

### 3.4.1 Mechanical transmission

The mechanical transmission of BSV was tried on different hosts (Table 2) with different buffers (Table 3). The compositions of the buffers used are given in (Appendix I).

**Table 2. Different hosts used for mechanical transmission**

| Sl.No | Common Name | Scientific Name |
|---|---|---|
| 1 | Ash gourd | *Benincasa hispida* Thunb. |
| 2 | Bitter gourd | *Momordica charanti* Descount. |
| 3 | Bottle gourd | *Lagenaria siceraria* (Molina) Standl. |
| 4 | Snake gourd | *Trichosanthes cucumerina* L. |
| 5 | Pumpkin | *Cucurbita pepo* L. |
| 6 | Cowpea | *Vigna unguiculata* (L.) Waln. |
| 7 | Colocasia | *Colacasia esculenta* (L.) Schott. |

The inoculum was prepared by grinding one part of young infected leaves of banana showing typical chlorotic streak symptoms with one part of pre-cooled buffer using a chilled pestle and mortar. The homogenate was filtered through a double layered muslin cloth. The extract was maintained in chilled condition. Prior to inoculation, the leaves of test plants were uniformly dusted with carborundum powder (600 mesh).The standard extract was then rubbed gently on

the leaves of test plants using cotton pad supporting the leaves with the other hand. After inoculation the leaves were washed with water using a wash bottle to remove any excessive inoculum and extraneous particles. The inoculated plants were kept in insect proof net house and observed for the development of symptoms.

**Table 3. Buffers used for mechanical transmission**

| Sl.No. | Buffers | pH |
|---|---|---|
| 1. | Sodium phosphate buffer | 7.2 |
| 2. | Potassium phosphate buffer | 7.2 |
| 3. | Borate buffer | 8.0 |
| 4. | Citrate buffer | 7.2 |
| 5. | Potassium phosphate and 2-mercaptoethanol (1%) | 7.2 |
| 6. | Tris buffer | 7.2 |

### 3.4.2 Soil transmission

Healthy tissue culture plants of banana variety Nendran (AAB) was raised in pots (size 24 cm × 72 cm) filled with the soil collected from the rhizosphere of BSV infected banana plants. These plants were kept in insect proof net house and observed for the development of symptoms. Healthy suckers raised in pots with the soil collected from disease free area were maintained as control (Plate 2).

### 3.4.3 Transmission through planting material

Transmission of BSV from mother plant to suckers was studied. The suckers of varieties, Chandrabale (AAB), Kalibale (AAB), Motta poovan (AAB), Mysore poovan (AAB) and Nendran (AAB) from both infected and healthy mother plants were collected and planted in pots. The plants were kept in insect

proof net house and expression of symptom was recorded at fortnightly intervals. The plants were periodically sprayed with insecticide to prevent vector transmission.

### 3.4.4 Insect transmission

The insect species used for the vector transmission studies of BSV were banana aphids (*Pentalonia nigronervosa* Coquerel) and mealy bugs like root mealybug (*Geococcus citrinus* Kuwana), pineapple mealy bug (*Dysmicoccus brevipes* Cockerell) and striped mealy bug (*Ferrisia virgata* Cockerell).

#### *3.4.4.1 Aphid transmission*

The banana aphid, *P. nigronervosa* Coquerel was used for the transmission studies.

##### *3.4.4.1.1 Collection and rearing of aphids*

The colony of banana aphids, *P. nigronervosa* Coquerel was reared and maintained on healthy banana suckers of variety Nendran (AAB) raised in pots and kept under shade in insect proof cages. These aphids were gently tapped and disturbed and then collected with the help of a camel hair brush in a Petri dish. After collecting the aphids, 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 camel hair brush for multiplication. Non-viruliferous aphids thus reared in insect proof cages were used for transmission studies (Plate 1a).

*3.4.4.1.2 Virus vector relationship*

*3.4.4.1.2.1 Effect of pre-acquisition fasting of P. nigronervosa on the transmission of BSV*

Group of 30 non-viruliferous *P. nigronervosa* Coquerel (both nymphs and adults) were fasted for various intervals of time viz., 0 hour, one hour, and two hours. A group of 30 unstarved aphids served as control. Then the aphids were allowed to feed on diseased plant for three days to acquire the virus. After the acquisition feeding period, the vectors were transferred to tissue culture banana plants of variety Nendran (AAB) for inoculation feeding. At the end of inoculation feeding period of seven hours, the aphids were killed by spraying 0.03 per cent dimethoate. The observations on development of symptoms were recorded.

*3.4.4.1.2.2 Effect of acquisition feeding of P. nigronervosa on transmission of BSV*

To determine the effect of acquisition feeding period on transmission, groups of 30 insects were starved for one hour and allowed to feed on infected plant for different periods viz., one day, two days, three days, and four days. At the end of acquisition feeding period, the aphids were transferred to tissue culture plants of variety Nendran (AAB) and later allowed to feed for seven hours for inoculation feeding. The insects were killed by spraying 0.03 per cent dimethoate and plants were kept in insect proof net house. The observations on development of symptoms were recorded.

## Plate 1a. Rearing of insects used in vector transmission

**Banana aphids**
**(*Pentalonia nigronervosa* Coquerel)**

**Pineapple mealy bug**
**(*Dysmicoccus brevipes* Cockerell)**

## Plate 1b. Rearing of insects used in vector transmission

**Striped mealy bug**
***(Ferrisia virgata* Cockerell)**

**Root mealy bug**
***Geococcus citrinus* Kuwana**

*3.4.4.1.2.3 Effect of inoculation feeding of P. nigronervosa on transmission of BSV*

To determine the minimum inoculation feeding period required to render the aphids viruliferous, groups of 30 insects were starved for one hour and allowed to feed on infected plant for three hours. At the end of acquisition feeding period the aphids were transferred to TC plants of variety Nendran (AAB) and allowed to feed for six hours, seven hours and eight hours. The insects were later killed by spraying dimethoate 0.03 percent and plants were kept under observation in insect proof net house. The observations on development of symptoms were recorded.

*3.4.4.2 Role of mealy bugs in the transmission of BSV*

Three different genera of mealy bugs viz., root mealy bug (*Geococcus citrinus* Kuwana), pineapple mealy bug (*Dysmicoccus brevipes* Cockerell) and striped mealy bug (*Ferrisia virgata* Cockerell) were used for the transmission studies.

*3.4.4.2.1 Collection and rearing of mealy bugs*

*3.4.4.2.1.a Collection and rearing of root mealy bugs-(Geococcus citrinus)*

The root mealy bugs (*G. citrinus* Kuwana) were collected from banana fields (Plate 1b). Rearing of mealy bugs was done on banana suckers. Banana suckers were planted in pots (size 24 cm×72 cm) and mealy bugs were released on the roots. This is to maintain the stock culture of the root mealy bug. After releasing the colonies the roots were covered with a thin layer of soil. The plants were irrigated daily to provide sufficient moisture for the survival of the root mealy bugs.

*3.4.4.4.1.b Collection and rearing of Pineapple mealy bug (Dysmicoccus brevipes)*

The pineapple mealy bugs were collected from different fields, brought to the laboratory and the technique of rearing the mealy bug culture on pumpkin under laboratory condition was standardized and validated. The matured fruits of pumpkin (*Cucurbita pepo* L.) without injury were used for rearing. These fruits were dipped in a solution of 0.1 per cent carbendazim for five minutes and air dried. Then the pumpkins were tied around with thread to provide grip to the crawling mealy bugs. The thread tied pumpkins were kept in ant pans. Once in a week, these pumpkins bearing the culture were exposed to the sun for drying up of the honey dew secreted by the mealy bugs and also to prevent fungal growth (Plate 1a).

*3.4.4.4.1.c Collection and rearing of Striped mealy bug - (Ferrisia virgata)*

The culture of *F. virgata* was reared on pumpkin (*Cucurbita pepo* L.) as given in 3.3.4.3.b.The attempts were also made to maintain the colonies of *F. virgata* Cockerell on plants of *Colacasia esculenta* (L.) Schott (Plate 1b).

*3.4.4.4 Role of Root mealy bug- Geococcus citrinus in the transmission of BSV*

*3.4.4.5 Virus – vector relationship*

Fifty numbers of root mealy bugs (*G. citrinus* Kuwana) including both adults and crawlers were collected from the banana fields. These mealy bugs were starved for one hour and these were released on the roots of infected banana to acquire the virus. Carefully these mealy bugs along with the soil were transferred to the roots of healthy tissue culture banana plants of variety Nendran (AAB). The inoculated plants were kept in the insect proof net house and observed for the development of symptoms.

*3.4.4.5.1 Effect of pre-acquisition fasting of D.brevipes and F. virgata on the transmission of BSV*

To determine the minimum acquisition-feeding period required to render the mealy bugs viruliferous, groups of thirty numbers of both *D.brevipes and F. virgata* were starved for zero hour, one hour and two hours. A group of 30 unstarved mealy bugs served as control. Then the insects were allowed to feed on diseased plant for 92 hours to acquire the virus. After the acquisition feeding period the vectors were transferred to banana plants, variety Nendran (AAB) for seven hours for inoculation feeding. At the end of inoculation feeding period the insects were killed by spraying 0.03 per cent dimethoate. The observations on development of symptoms were recorded.

*3.4.4.5.2 Effect of acquisition feeding of D.brevipes and F.virgata on the transmission of BSV*

To determine minimum acquisition feeding period required to render the mealy bugs viruliferous, groups of 30 insects were starved for one hour and allowed to feed on infected plant for different periods viz., 24 hours, 48 hours, 92 hours and 116 hours. At the end of acquisition feeding period the mealy bugs were transferred to healthy tissue culture banana variety Nendran (AAB) and allowed to feed for seven hours for inoculation feeding. The mealy bugs were killed later by spraying 0.03 per cent dimethoate and plants were kept under observation in insect proof net house. The observations on development of symptoms were recorded.

*3.4.4.5.3 Effect of inoculation feeding of D.brevipes and F.virgata on the transmission of BSV*

To determine the minimum inoculation feeding period required to render the mealy bugs viruliferous, groups of 30 insects were starved for one hour. Then the vectors were allowed to feed on diseased plant parts for three days to acquire the virus. After acquisition feeding period the vectors were transferred to banana plants, variety Nendran (AAB) for six hours, seven hours and eight hours for inoculation feeding. At the end of inoculation feeding the insects were killed by spraying 0.03 per cent dimethoate. The observations on development of symptoms were recorded.

*3.4.4.5.4 Effect of number of mealy bugs, D.brevipes and F.virgata in the transmission of BSV*

To determine the minimum number of mealy bugs which could transmit the virus a group of 10, 20, 30, 40 numbers of viruliferous mealy bugs were starved for one hour and allowed to feed on BSV infected plants for 92 hours. After the acquisition feeding period the mealy bugs were transferred to banana plants, variety Nendran (AAB) plants and allowed to feed for seven hours. The plants were later sprayed with 0.03 per cent dimethoate to kill the viruliferous vectors. The observations on development of symptoms were recorded.

## 3.5 ELECTRON MICROSCOPY

Electron microscopic investigation of the infected leaf samples was done to study the morphology of the BSV particles. This work has been done utilizing the National diagnostic facility of IIHR, Bangalore. Symptomatic leaf tissue (3mm diam) of banana was crushed over a clean glass slide in 0.01M phosphate buffer (pH 6.5). Homogenate (10µl) was placed on a parafilm and EM carbon coated grid (film side downward) was floated on the surface of the droplet for 10

minutes. The grid was then removed and washed with double distilled water. 2-4 drops of 2% uranyl acetate (UA) were added on the filmed surface of the grid. Excess stain was removed and the grid was left to dry. Later this was viewed under electron microscope.

## 3.6 MOLECULAR DIAGNOSIS OF BANANA STREAK VIRUS USING PCR

The molecular diagnosis of Banana streak virus was carried out in the molecular virology laboratory of Banana Research station, Kannara. Infected samples of banana varieties Nendran (AAB), Mysore poovan (AAB) and a healthy control were taken for indexing.

### 3.6.1 Isolation of DNA

DNA was isolated from leaves of infected plants showing typical chlorotic and necrotic streaks using DNeasy plant minikit (Qiagen, Gmbh, Hilden, Germany) as per the protocol provided by manufacturer. The DNA was also isolated from the leaves of healthy plants to serve as control. The varieties used for DNA isolation were Nendran (AAB) and Mysore poovan (AAB).

### 3.6.2 Agarose Gel Electrophoresis

Agarose gel electrophoresis was performed based on the method described by Sambrook *et al.* (1989) to check the quality of DNA, under sterile conditions.1X TAE buffer was prepared from the 50X (pH 8.0) stock solutions (Appendix II). Agarose (Genei, Low EEO) (1 percent) was weighed and dissolved in TAE buffer by melting. Ethidium bromide prepared from a stock of 10mg/ml was added to it, at a concentration of 0.5µg/ml and mixed well. The open end of the gel casting tray was sealed with cello tape and the tray was placed on a horizontal surface. The comb was placed properly and the dissolved agarose was poured into the tray. The gel was allowed to set for 30 minutes and later the comb

was removed carefully. The gel was then placed in the electrophoresis unit with the well side directed towards the cathode.1X TAE buffer was added to the buffer tank (GeNei, Banglore) so as to cover the gel with a few mm of buffer. 5µl of DNA sample was mixed with 1µl tracking dye (6X) (Appendix III) and carefully loaded into the wells using a micropipette. The cathode and the anode of the electrophoresis unit were connected to the power pack (GeNei, Banglore) and the gel was run at constant voltage of 70V (35mA) for 75 minutes. The power was turned off when the tracking dye reached about 3cm from the anode end.

### 3.6.3 Gel documentation

The DNA bands separated by electrophoresis were viewed and photographed using Alpha Imager TM 1200 documentation and analysis system.

### 3.6.4. Purity of DNA

The purity of DNA was further analysed by using NanoDrop® ND-1000 spectrophotometer. This is a full spectrum (220-750nm) spectrophotometer that measures 1µl samples with high accuracy and reproducibility.

The absorbance of nucleic acid samples was measured at a wavelength of 260nm and 280nm. The ratio (260/280) was used to assess the purity of nucleic acids.

## 3.7 POLYMERASE CHAIN REACTION

Polymerase chain reaction of BSV was done using specific primers designed by Cherian *et al.* (2004). Specific primers were designed from the region corresponding to the conserved domain of Reverse transcriptase and RNase H gene. The forward primer used was BSV 5466-5'AGAGTGGGTTTCATCAAGTAGC and reverse primer was BSV 6196-5'

GAATTTCCCGCTCGCATAAG. The primer number indicates the position on BSV genome.

### 3.7.1 Standardization of PCR conditions

Various combinations of PCR parameters like annealing temperature and template dilution were tested to find out the optimum combination of conditions, varying one parameter at a time. Annealing temperatures of 57°C, 58°C, 59°C, 60°C, 61°C, 62°C and 63°C and different template dilutions of 1:10, 1:25, 1:50, 1:100 and undiluted DNA were used for the standardization

### 3.7.2 PCR amplification using standardized condition

Polymerase chain reaction was carried out in standardized conditions (annealing temperature-59, template dilution-1/10) using specific primers given in the section 3.7. The PCR products were electrophoresed in 1% agarose gel and documented and compared with 1Kb DNA marker (GeNei,Banglore). The composition of the reaction mixture used for PCR is given below

| Items | Volume (µl) |
|---|---|
| 10x PCR buffer with $MgCl_2$ (GeNei,Banglore) | 2.5 |
| Taq DNA polymerase (0.05U/ µl) (GeNei,Banglore) | 1 |
| dNTP mix (2mM) (Fermentas, Lithuania) | 5 |
| Forward primer (100µM)(GeNei,Banglore) (1:10) | 1 |
| Reverse primer (100µM) (GeNei,Banglore) (1:10) | 1 |
| Template DNA (1:10) | 5 |
| Sterile water | 34.5 |
| Total | 50.0 |

The reaction mixture dispensed in 0.2ml tubes was given a momentary spin for thorough mixing of the cocktail components. The PCR tubes were then placed in a thermalcycler (Eppendorf Mastercycler Gradient).

The PCR was carried out by following steps

Step 1: Initial denaturation at 94°C for 4 minutes

Step 2: Denaturation at 94°C for 30 seconds
Step 3: Annealing at 59°C for 30 seconds
Step 4: Extension at 72°C for 30 seconds
} 30 cycles

Step 5: Final extension at 72°C for 10 minutes

Step 6: 4°C for 5 minutes to hold the sample

### 3.8. Immunocapture-PCR

A more sensitive and reliable technique *viz.*, IC-PCR was also standardized. Immuno-capture was standardized using the antiserum raised against Banana streak virus which was procured from AGDIA.

Immunocapture PCR was performed using a BSOLV antiserum raised against the purified BSV species. Sterile propylene thin walled 0.2 ml microfuge tubes were coated with 50 µl of IgG, diluted at 1:200 ratios in the carbonate coating buffer for four hours at room temperature. Then the tubes were washed three times with 100 µl of PBS-T washing buffer. Plant extract was prepared by grinding 1gm of leaf sample in 5ml of the coating buffer using a sterile ice cold pestle and mortar. These extracts were transferred to sterile 1.5ml tubes and centrifuged at room temperature for 10 minutes at 5000 rpm. 50µl of the supernatant were loaded in the IgG coated tubes and incubated overnight at 5°C.

The tubes were washed three times using 100µl PBS-T buffers and rinsed with sterile water. DNase treatment was done to degrade the host genome if present in the tubes. For this 3U of DNase in 200µl freshly prepared buffer was added and inocubated at room temperature for 30 minutes. The tubes were washed

three times with 100µl PBS-T buffer and rinsed one time with sterile water. The PCR mastermix was prepared and added to the tubes. The composition of the buffers is listed in (Appendix IV). The PCR tubes were then placed in a thermalcycler (Eppendorf Mastercycler Gradient). The PCR products were electrophoresed in 1.5 % agarose gel and documented.

The composition of the reaction mixture used for PCR is given below

| Items | Volume (µl) |
|---|---|
| 10x PCR buffer with $MgCl_2$ (GeNei,Banglore) | 2.5 |
| Taq DNA polymerase(3U/µl) (1:10) (Genei,Banglore) | 5 |
| dNTPmix (0.2mM) (Genei,Banglore) | 0.5 |
| Forward primer (100µM)(1:10) (GeNei,Banglore) | 1 |
| Reverse primer (100µM) (1:10) (GeNei,Banglore) | 1 |
| Sterile water | 15 |
| Total | 25.0 |

The PCR was carried out by many steps

Step 1: Initial denaturation at 94°C for 30 seconds

Step 2: Denaturation at 94°C for 20 seconds

Step 3: Annealing at 59°C for 30 seconds } 30 cycles

Step 4: Extension at 72°C for 60 seconds

Step 5: Final extension at 72°C for 3 minutes

Step 6: 4°C for 5 minutes to hold the sample

# Results

# 4. RESULTS

## 4.1 COLLECTION AND MAINTENANCE OF BSV INFECTED PLANTS

The BSV infected suckers maintained at BRS, Kannara under insect proof net house served as the source of inoculum. Apart from these, naturally infected suckers of different banana varieties such as Chandrabale (AAB), Kalibale (AAB), Motta poovan (AAB), Mysore poovan (AAB) and Nendran (AAB) were collected from different fields. These were planted in pots and also maintained in the insect proof net house.

## 4.2 SYMPTOMATOLOGY

Symptomatology was studied by assessing the development of symptoms on infected plants as given in 3.2.

### 4.2.1 Symptoms on leaf lamina

The symptoms were mostly seen on the leaf lamina (Plate 2a). Initially the symptoms appeared as discontinuous or continuous linear small chlorotic streaks on the leaf lamina. These chlorotic streaks later turned necrotic, blackened and running perpendicular to the leaf axis extending from midrib to leaf margin or sometimes form a linear mosaic like pattern on the lamina especially on older leaves. During summer months apart from chlorotic and necrotic streaks, thickening and distortion of leaf lamina were seen (Plate 2b). Scoring of the severity of symptoms on leaves was done. The development of symptoms was same in all BSV infected varieties. The expression of the symptoms was more in the months of June-December and less during January-May seasons. In advanced stages, there was necrosis of cigar leaf and the plant failed to produce new leaves and resulted in death of the plant.

**Plate 2a. Symptoms on leaf lamina**

**i) Chlorotic streaks**

**ii) Mosaic like appearance**

## Plate 2b. Symptoms on leaf lamina

i) Necrotic streaks

ii) Distortion of leaf lamina

iii) Cigar leaf necrosis

**Plate 3. Symptoms on midrib and petiole**

**i) Necrotic streaks on midrib**

**ii) Necrotic streaks on petiole**

**Plate 4. Symptoms on pseudostem and bunch**

i) Necrotic streaks on pseudostem

ii) Necrotic streaks -C.S of pseudostem


iii) Infected bunches

### 4.2.2 Symptoms on mid rib and leaf petiole

Small dark brown lesions appeared on the petiole and later joined to form linear elongated lesions. The length of the lesions ranges from 0.1cm-0.3cm. Such lesions were seen on the leaf petiole of banana varieties Motta poovan (AAB), Mysore poovan (AAB), Kalibale (AAB) and Nendran (AAB) (Plate 3).

### 4.2.3 Symptoms on pseudostem

Small dark brown necrotic streaks were seen on the pseudostem of BSV infected plants. The pseudostem streaks were seen on varieties viz., Mysore poovan (AAB), Motta poovan (AAB), Kalibale (AAB) and Nendran (AAB). Initially it appeared as dark or brown linear streaks, which later developed into continuous dark streaks (Plate 4).

### 4.2.4 Symptoms on bunches

Infected plants produced small sized bunches with distorted fingers. Brown and necrotic streaks were developed on the fruits in the case of severe infection of the disease. Severe infection failed to produce bunches as in the case of Nendran (AAB), Mysore poovan (AAB) and Chandrabale (AAB) varieties (Plate 4).

## 4.3 IMPACT OF THE DISEASE ON THE GROWTH PARAMETERS

### 4.3.1 Height of pseudostem

The mean values of the height of the plant of different varieties at 6 MAP (months after planting) are given in Table 4. The results showed that the BSV infection reduced plant height. The per cent reduction in plant height was the highest in the variety Kalibale (19.37) and lowest in Chandrabale (8.71).

**Table 4. Effect of BSV infection on biometric characters**

| Banana variety | Height (cm) | | | Girth (cm) | | | Number of leaves | | |
|---|---|---|---|---|---|---|---|---|---|
| | Healthy | Infected | Per cent reduction | Healthy | Infected | Per cent reduction | Healthy | Infected | Per cent reduction |
| Mottai poovan(AAB) | 215.4 | 190.2 | 11.69 | 50.76 | 45.3 | 10.71 | 10.3 | 9.2 | 10.67 |
| Mysore poovan(AAB) | 213.6 | 172.3 | 19.35 | 51.46 | 42.2 | 17.95 | 11.2 | 8.2 | 26.78 |
| Kalibale(AAB) | 217.3 | 175.2 | 19.37 | 50.24 | 40.3 | 19.36 | 9.2 | 7.3 | 20.65 |
| Nendran(AAB) | 230.6 | 198.5 | 13.92 | 48.54 | 42.5 | 12.44 | 11.3 | 10.2 | 9.73 |
| Chandrabale(AAB) | 214.5 | 195.8 | 8.71 | 51.62 | 47.2 | 8.50 | 10.4 | 9.6 | 9.52 |

### 4.3.2 Girth of pseudostem

The girth of pseudostem at 20cm above the ground level was recorded. The results revealed that the virus infection adversely affected the girth of the plant (Table 4). Highest per cent reduction was in Kalibale (19.36) and lowest in the variety Chandrabale(8.50).

### 4.3.3 Number of leaves

The results indicated that there was a reduction in the number of leaves of diseased plants compared to healthy plants (Table 4). The per cent reduction was maximum in the variety Mysore poovan (26.78) and the lowest in the variety Chandrabale (9.52).

### 4.3.4 Bunch Weight

Yield reduction was observed due to virus infection. The per cent reduction in bunch weight due to infection was 45.98 and 22.6 respectively in varieties Kalibale (AAB) and Motta poovan (AAB).The bunch weight of infected and healthy plants are given in table 5a & 5b.The yield characters of the varieties, Motta poovan (AAB) and Kalibale (AAB) and are presented below. The varieties, Nendran (AAB), Chandrabale (AAB) and Mysore poovan (AAB) did not produce bunches.

### 4.3.5 Number of hands per bunch

Reduction in number of hands was observed due to virus infection. The percentage reduction in number of hands due to BSV infection was 9.67 and 29.78 in banana varieties, Mottapoovan (AAB) and Kalibale (AAB) respectively (Table 5a).

**Table 5a. Effect of BSV infection on yield characters**

| Banana variety | Bunch weight (Kg) | | | Number of hands per bunch | | | Number of fingers | | |
|---|---|---|---|---|---|---|---|---|---|
| | Healthy | Infected | Per cent reduction | Healthy | Infected | Per cent reduction | Healthy | Infected | Per cent reduction |
| Motta poovan(AAB) | 10.4 | 8.08 | 22.60 | 10.2 | 9.3 | 9.67 | 180 | 150 | 16.66 |
| Kalibale(AAB) | 7.22 | 3.90 | 45.98 | 7.4 | 5.2 | 29.78 | 94 | 63 | 32.97 |

**Table 5b.Effect of BSV infection on fruit characters**

| Banana variety | Fruit weight(gm) | | | Length of finger(cm) | | | Girth of finger (cm) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Healthy | Infected | Per cent reduction | Healthy | Infected | Per cent reduction | Healthy | Infected | Per cent reduction |
| Motta poovan(AAB) | 44.53 | 29.25 | 34.31 | 8.32 | 6.48 | 22.11 | 11.35 | 8.32 | 26.69 |
| Kalibale(AAB) | 65.58 | 25.22 | 61.54 | 11.52 | 7.28 | 36.80 | 10.68 | 8.52 | 20.22 |

### 4.3.6 Number of fingers per bunch

Virus infection reduced the number of fingers per bunch. The per cent reduction due to infection was 16.66 and 32.97 respectively in banana varieties, Motta poovan (AAB) and Kalibale (AAB) (Table 5a).

### 4.3.7 Weight of the fruit

The weight of the fruit was reduced due to virus infection. The per cent reduction due to infection was 34.31 and 61.54 respectively on banana varieties, Motta poovan (AAB) and Kalibale (AAB) (Table 5b).

### 4.3.8 Length of the finger

The length of the finger was reduced due to virus infection. The per cent reduction in the finger length over healthy control was 22.11 and 36.80 respectively on Motta poovan (AAB) and Kalibale (AAB) (Table 5b).

### 4.3.9 Girth of the finger

There was reduction in the finger girth due to the virus infection. Results showed that the percent reduction was 26.69 and 20.22 respectively in banana varieties Mottapoovan (AAB) and Kalibale (AAB) (Table 5b).

### 4.4 Seasonal variability in expression of symptoms

A correlation analysis was carried out between the weather parameters and disease incidence. The weather data is given in Appendix III. Significant correlation was observed between the expression of symptom with rainfall, temperature and humidity. The symptom expression was more during cool seasons when there is heavy rainfall. A positive correlation was observed between

**Table 6. Effect of weather parameters on symptom expression of BSV**

| Banana variety | Rainfall(mm) | Temperature(° C) | Humidity (%) |
|---|---|---|---|
| Kalibale (AAB) | 0.365** | -0.119* | 0.309 |
| Motta poovan(AAB) | 0.277** | -0.713** | 0.166 |
| Mysore poovan(AAB) | 0.117** | -0.615** | 0.116* |

**Significance level at 0.01 %, *Significance level at 0.05 %

**Table 7. Occurrence of BSV in the accessions of the field gene bank**

| Ploidy | Number of cultivars infected | Name of the accession |
|---|---|---|
| AA | 0 | - |
| AAA | 0 | - |
| AAB | 6 | Motta poovan<br>Mysore poovan<br>Kalibale<br>Chandrabale<br>Chinali, Nendran. |
| ABB | 0 | - |
| AAAB | 1 | FHIA-3 |

**Table 8. Incidence and banana streak disease on different varieties of banana**

| Banana variety | PDS | PDI (%) | CI | SSI |
|---|---|---|---|---|
| Mysore poovan(AAB) | 32.16 | 100 | 32.16 | 40.88 |
| Kalibale(AAB) | 31.22 | 100 | 31.22 | 31.34 |
| Nendran(AAB) | 16.09 | 60 | 9.65 | 20.98 |
| Mottapoovan(AAB) | 23.44 | 100 | 23.44 | 35.16 |
| Chandrabale(AAB) | 14.89 | 40 | 5.98 | 18.31 |
| Chinali(AAB) | 12.25 | 20 | 2.65 | 13.25 |
| FHIA-3(AAAB) | 30.35 | 20 | 6.07 | 15.22 |

PDS-Percent disease severity CI-Coefficient of infection

PDI-Percent disease incidence SSI-Symptom severity index

**Table 9. Transmission of BSV through planting materials**

| Banana variety | Mean number of suckers per plant | Mean number of infected suckers | Percentage of infected suckers (%) |
|---|---|---|---|
| Mysore poovan(AAB) | 4.4 | 3.8 | 86.36 |
| Kalibale(AAB) | 4.2 | 2.8 | 66.66 |
| Nendran(AAB) | 4.0 | 1.2 | 30.00 |
| Motta poovan(AAB) | 4.2 | 4.0 | 95.23 |
| Chandrabale(AAB) | 3.6 | 0.6 | 16.66 |

symptom expression and rainfall. Like rainfall, humidity also showed positive correlation with symptom expression. A negative correlation was observed between expression of symptoms and temperature (Table 6).The symptom expression was less during hot seasons.

## 4.5 SCREENING OF FIELD GENE BANK

Banana accessions maintained in the field gene bank of Banana Research Station, Kannara were screened at monthly intervals for their reaction to the disease (Plate 5). A total of 290 accessions were screened and out of which seven accessions were found to be infected (Table 7). The study revealed that the accessions with only 'A' genome were comparatively resistant compared to those with 'B' genome.

The symptoms were discrete chlorotic streaks, spindle shaped chlorotic streaks, long streaks running parallel to lamina. Later chlorotic streaks turned necrotic. In the case of hybrid variety, FHIA-3 symptoms were seen as necrotic streaks inside the pseudostem. The symptoms of BSV expressed by different accessions in the germplasm was showed in (Plate 6)The severely infected plants fail to flower and death of the plant occurred. Based on the scoring data the Percent disease incidence (PDI), Percent disease severity (PDS), Coefficient of infection (CI) and Symptom severity index (SSI) were calculated and presented in the Table 8.

The percent disease severity was highest in the variety Mysore poovan (32.16) and least Chinali (13.25). The percent disease incidence was 100 per cent in varieties Mysore poovan, Kalibale and Motta poovan. The coefficient of infection was highest Mysore poovan (32.16) and lowest in Chinali (2.65). The symptom severity index was the highest Mysore poovan (40.88) and least in Chinali (13.25).

**Plate 5. Field gene bank maintained at Banana Research Station, Kannara**

**Plate 6. Symptoms of banana streak disease on different accessions of field gene bank**

i) Nendran(AAB)


ii) Mottapoovan(AAB)

iii) Kalibale (AAB)


iv) Chandrabale (AAB)

v)Mysore poovan(AAB)

## 4.6 TRANSMISSION STUDIES

The transmission studies were carried out under glass house condition.

### 4.6.1 Mechanical Transmission

Results of mechanical transmission studies showed that BSV could not be transmitted mechanically from infected banana to other host plants viz., cucurbits, cowpea and colocasia.

### 4.6.2 Soil Transmission

Transmission of the virus was done in tissue culture plants raised in pots filled with the soil collected from the rhizosphere of BSV infected banana plants. None of the plants were found to be infected, thus confirming that disease is not transmitted through soil.

### 4.6.3 Transmission through suckers

The results showed that the virus is transmitted through the suckers of infected plants. The rate of transmission through the sucker is presented in the Table 9. The variety Motta poovan (AAB) recorded the highest per cent transmission (95.23) and Chandrabale (AAB) recorded the lowest (16.66). The sucker transmission recorded in other varieties such as Kalibale (AAB), Nendran (AAB), Mottapoovan (AAB) and Chandrabale (AAB) were 66.66, 30, 95.23 and 16.66 per cent respectively.

### 4.6.4 Virus-vector relationship - Banana aphids

Results of the studies on virus-vector relationship using banana aphid, *Pentalonia nigronervosa* showed that the virus is not transmitted by banana

aphids. None of the plants inoculated with viruliferous aphids produced the symptoms of banana streak disease.

### 4.6.5 Virus vector relationship - Pineapple mealy bug

#### *4.6.5.1 Effect of pre-acquisition fasting on the transmission of BSV*

Fasting of both nymphs and adults of *Dysmicoccus brevipes* Cockerell prior to acquisition access feeding had no significant effect on the percent transmission of BSV. The per cent transmission recorded was 13.33, when nymphs and adults were given a pre-acquisition fasting of 1hour and 2 hours (Table 10).

#### *4.6.5.2 Effect of acquisition feeding on the transmission of BSV*

Results showed that nymphs and adults required a minimum period of three days for acquiring the virus from source plant. When four days acquisition feeding period was given virus transmission could not be obtained.

#### *4.6.5.3 Effect of inoculation access period on the transmission of BSV*

A minimum period of seven hours was required for the successful inoculation of the virus. The per cent transmission was 13.33 in the case of 7 and 8 hours (Table 12).

#### *4.6.5.4 Effect of number of mealy bugs on the transmission of BSV*

The minimum number of mealy bugs required for the transmission of BSV was observed to be 30. The rate of transmission was 13.33 percent. Transmission rate was increased when the number of viruliferous mealy bugs used for transmission was increased (Table 13). The microscopic view of mealy bugs were showed in the Plate 7.The symptoms were expressed as small discontinuous

chlorotic streaks on the leaf lamina (Plate 8).The symptoms were produced four weeks after inoculation.

### 4.6.6 Virus vector relationships - Striped mealy bugs

#### *4.6.6.1. Effect of pre-acquisition fasting on the transmission of BSV*

Fasting of both nymphs and adults of *Ferrisia virgata* Cockerell prior to acquisition access feeding had significant effect on the percent transmission of BSV. The transmission obtained was 6.66 per cent when the mealy bugs were given a pre-acquisition fasting of one hour and two hours prior to acquisition feeding (Table 14).

#### *4.6.6.2. Effect of acquisition feeding on the transmission of BSV*

Results showed that a period of three days was required for the acquisition of virus from source plant. The rate of transmission was 6.66 percent (Table 15).

#### *4.6.6.3. Effect of inoculation access period on the transmission of BSV*

The study on the effect of inoculation access period on the efficiency of transmission of BSV showed that a minimum period of seven hours was required for successful inoculation of the virus. The percent of transmission was 6.66. (Table 16).

#### *4.6.6.4. Effect of number of mealy bugs in the transmission of BSV*

It is obvious that a minimum of 30 numbers of mealy bugs were required for the transmission of BSV. Transmission rate was increased when the numbers of viruliferous mealy bugs used for inoculation were increased (Table 17).The rate of transmission was 6.66 per cent. The microscopic view of mealy bugs was

**Table 10. Effect of pre- acquisition fasting period of *Dysmicoccus brevipes* on the transmission of BSV**

| Pre-acquisition fasting period (Hours) | No. of plants inoculated | No. of plants infected | Percentage of transmission (%) |
|---|---|---|---|
| 0 | 15 | 0 | 0 |
| 1 | 15 | 2 | 13.33 |
| 2 | 15 | 2 | 13.33 |

**Table 11. Effect of acquisition feeding of *Dysmicoccus brevipes on* the transmission of BSV**

| Acquisition feeding period (Days) | No of plants inoculated | No of plants infected | Percentage of transmission (%) |
|---|---|---|---|
| One | 15 | 0 | 0 |
| Two | 15 | 0 | 0 |
| Three | 15 | 2 | 13.33 |
| Four | 15 | 0 | 0 |

**Table 12. Effect of inoculation access period of *Dysmicoccus brevipes* on the transmission of BSV**

| Inoculation access period (Hours) | No of plants inoculated | No of plants infected | Percentage of transmission (%) |
|---|---|---|---|
| 6 | 15 | 0 | 0 |
| 7 | 15 | 2 | 13.33 |
| 8 | 15 | 2 | 13.33 |

**Table 13. Effect of number of *Dysmicoccus brevipes* on the transmission of BSV**

| Number of mealy bugs (Numbers) | No of plants inoculated | No of plants infected | Percentage of transmission (%) |
|---|---|---|---|
| 10 numbers | 15 | 0 | 0 |
| 20numbers | 15 | 0 | 0 |
| 30numbers | 15 | 2 | 13.33 |
| 40numbers | 15 | 2 | 13.33 |

**Table 14.Effect of pre-acquisition fasting period of *Ferrisia virgata* on the transmission of BSV**

| Pre-acquisition fasting period (Hours) | No of plants inoculated | No of plants infected | Percentage of transmission (%) |
|---|---|---|---|
| 0hour | 15 | 0 | 0 |
| 1hour | 15 | 1 | 6.66 |
| 2hours | 15 | 1 | 6.66 |

**Table 15. Effect of acquisition feeding of *Ferrisia virgata on* the transmission of BSV**

| Acquisition feeding period (Hours) | No of plants inoculated | No of plants infected | Percentage of transmission (%) |
|---|---|---|---|
| 24hours | 15 | 0 | 0 |
| 48hours | 15 | 0 | 0 |
| 72hours | 15 | 1 | 6.66 |
| 96hours | 15 | 0 | 0 |

**Table 16. Effect of inoculation access period of *Ferrisia virgata* on the transmission of BSV**

| Inoculation access period (Hours) | No of plants inoculated | No of plants infected | Percentage of transmission (%) |
|---|---|---|---|
| 6hours | 15 | 0 | 0 |
| 7hours | 15 | 1 | 6.66 |
| 8hours | 15 | 1 | 6.66 |

**Table 17. Effect of number of *Ferrisia virgata* on the transmission of BSV**

| Number of mealy bugs (Numbers) | No of plants inoculated | No of plants infected | Percentage of transmission (%) |
|---|---|---|---|
| 10numbers | 15 | 0 | 0 |
| 20 numbers | 15 | 0 | 0 |
| 30 numbers | 15 | 1 | 6.66 |
| 40 numbers | 15 | 1 | 6.66 |

## Plate 7. Mealy bugs used in vector transmission

**Pineapple mealy bug**
**Adult-40x**

**Pineapple mealy bug**
**Nymphs-20x**

**Striped mealy bug**
**Adult-40x**

**Striped mealy bug**
**Nymphs-40x**

**Root mealy bug**
**Adult-40x**

**Plate 8. Symptoms developed after vector transmission**

**Plate 9. Banana streak virus particles under electron microscope**

showed in the Plate 7. The symptom expressed as small discrete chlorotic streaks (Plate 8). The symptoms were produced six weeks after transmission.

**4.6.7 Virus - vector relationship - *Geococcus* sp.**

In order to study the role of root mealy bug *Geococcus* sp. as the vector of BSV, transmission was carried out in tissue culture plants of banana variety Nendran (AAB) using viruliferous mealy bugs .The inoculated plants did not produce any symptoms. The microscopic view of mealy bugs were showed in Plate 7.

4.7 ELECTRON MICROSCOPY

The electron microscopic observations of infected leaf of banana showed typical bacilliform particles, of size 130-150 × 30nm.Thus confirming that the streak like symptoms in banana are associated with the infection of BSV (Plate 9).

4.8 MOLECULAR DIAGNOSIS OF BANANA STREAK VIRUS BY PCR

The polymerase chain reaction was carried out using specific primers for the detection of BSV. Specific primers were designed from the region corresponding to the conserved domain of reverse transcriptase and RNase H.

The DNA of the two isolates and negative control (healthy) were isolated using DNeasy plant minikit (Qiagen, Gmbh, Hilden, Germany) by the protocol provided by them. The isolated DNA was visualized as three intact bands obtained on electrophoresis of the product using 0.8 per cent agarose gel (Plate 10).

### 4.8.1 Estimation of quantity and quality of DNA

The quantity and quality of DNA were assessed by spectrophotometry and was given in Table 18. The ratio, $OD_{260/280}$ ranged from 1.69 to 2.13.

## 4.9. STANDARDIZATION OF PCR CONDITIONS

PCR conditions such as specific primer combinations, annealing temperature, and template dilution were standardized. The amplified product was of size 730bp.

### 4.9.1. Effect of annealing temperature on PCR amplification in the primer combination BSV5466 and BSV6196

Different annealing temperatures ranging from 57-63°C were kept for PCR amplification of BSV. The intensity of amplification was the highest at 59°C (Plate 10). The amplification was obtained at the temperature at 57° C, 58° C, 60°C, 61° C, 62° C and 63° C but with low intensity (Table 19).

### 4.9.2 Effect of template dilution on PCR amplification in the primer combination BSV 5466 and BSV 6196

Different template dilutions were prepared from DNA sample of concentration of 272.2ng/µl. The dilutions were 1:10, 1:25, 1:50, and 1:100.The intensity of amplification was found to be maximum at 1:10 dilution (Plate 10).The intensity was low at other dilutions (Table 20).

**Table 18. Quality and quantity of DNA synthesized from healthy and infected samples**

| DNA sample | Ratio OD260/OD280 | Quantity of DNA ng/µl |
|---|---|---|
| Mysore poovan (AAB) | 1.79 | 54.4 |
| Nendran (AAB) | 1.78 | 47.8 |
| Healthy sample | 1.47 | 40.7 |

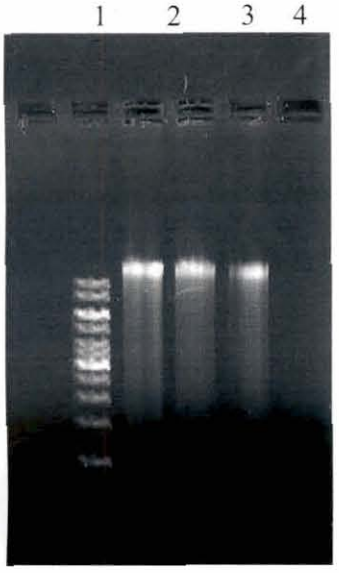
**Table 19. Effect of annealing temperature on PCR amplification in the primer combination BSV5466 and BSV6196**

| Primer combination | Annealing Temperature | | | | | | |
|---|---|---|---|---|---|---|---|
| | 57°C | 58°C | **59°C** | 60°C | 61°C | 62°C | 63°C |
| BSV 5466 and BSV 6196 | Amplified product with low intensity | Amplified product with low intensity | **Amplified product with good intensity** | Amplified product with low intensity | Amplified product with low intensity | Amplified product with low intensity | Amplified product with low intensity |

**Table 20. Effect of template dilution on PCR amplification in the primer combination BSV5466 and BSV6196**

| Primer combination | Template dilution | | | | | |
|---|---|---|---|---|---|---|
| | 272.4ng | **1:10** | 1:25, | 1:50 | 1:75 | 1:100 |
| BSV 5466 and BSV 6196 | Amplified product with low intensity | **Amplified product with good intensity** | Amplified product with low intensity | Amplified product with low intensity | Amplified product with low intensity | Amplified product with low intensity |

## Plate 10. Molecular detection of Banana streak virus using PCR

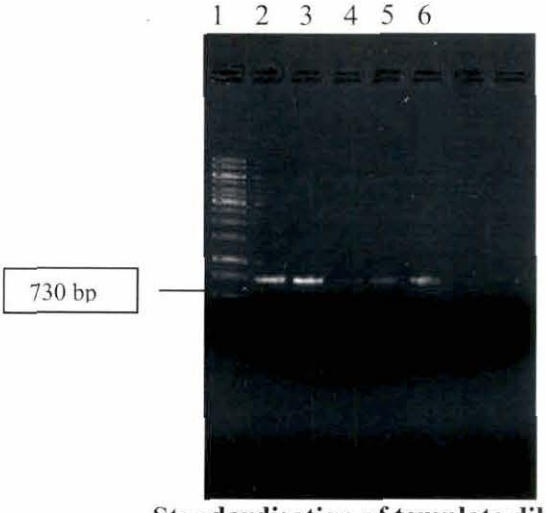



**DNA isolation**

Lane 1 - 1 kb DNA marker
Lane 2 - Mysore poovan (AAB)
Lane 3 - Nendran (AAB)
Lane 4 - Control


**Standardisation of annealing temperature**

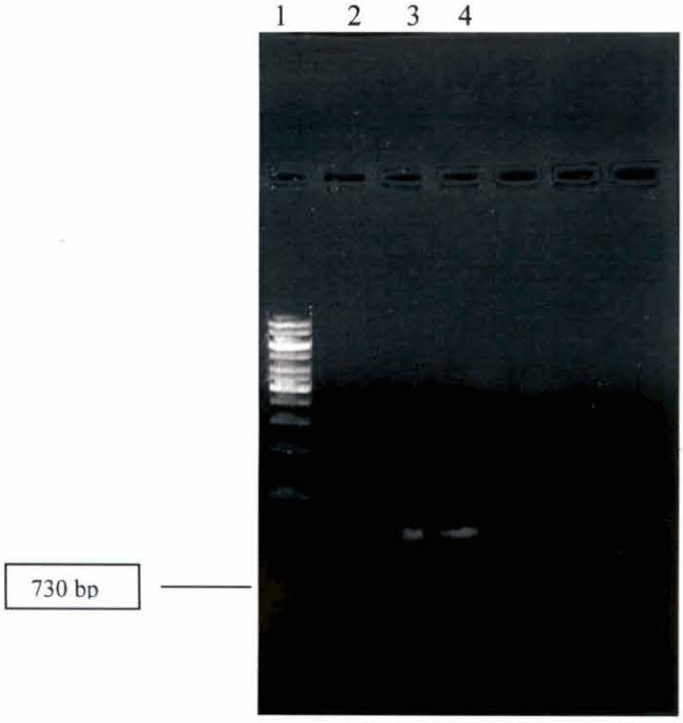
Lane 1 - 1 kb DNA marker
Lanes 2 – 8 - 57 °C-63 °C




**Standardisation of template dilution**

Lane 1-1kb DNA marker
Lane 2-Concentrated DNA sample 272.4 ng/µl
Lane 3-1:10
Lane 4-1:25
Lane 5-1:50
Lane 6-1:100

**Plate 11. Molecular detection of Banana streak virus using IC-PCR**




Lane 1 -1kb DNA marker
Lane 2 - Control
Lane 3&4 - BSV infected sample Mysore poovan(AAB)

## 4.10 POLYMERASE CHAIN REACTION WITH STANDARDIZED CONDITIONS

The primer combination BSV5466 and BSV6196 yielded an amplified product of 730bp size. The isolated DNA of BSV isolates, Nendran (AAB) and Mysorepoovan (AAB) were used for amplification. PCR was carried out with the primers at an annealing temperature of 59° C and a template dilution of 1:10 along with primer concentration of 1:10. The electrophoresis of the amplicons showed clear bands of approximate size 730bp. Amplification was not obtained in healthy control.

## 4.11 IMMUNO CAPTURE -PCR

IC-PCR was carried out with the primers at an annealing temperature of 59°C and primer dilution of 1:10. The BSV infected isolates of Mysore poovan (AAB) was used for the amplification. The primer combination BSV5466 and BSV6196 yielded an amplified product of 730bp (Plate 11). Amplification was not observed in healthy control.

# *Discussion*

# 5. DISCUSSION

Bananas and plantains (*Musa* spp.) are grown as a staple food, a significant cash crop and a major export crop in many of the tropical and subtropical countries of the world including India (Annual Report INIBAP, 1992). They are affected by four viral diseases viz., banana bract mosaic virus (BBrMV), banana bunchy top virus (BBTV), banana streak virus (BSV) and cucumber mosaic virus (CMV) (Burns *et al.*, 1994; Diekmann and Putter, 1996 and Thomas *et al.*, 1998). These viruses pose a threat to banana production in areas where the viruses are endemic. No effective resistance is known in *Musa* to any of these viruses, so control is still largely based on the use of virus free planting material, rouging of infected plants, implementation of quarantine barriers etc. All these viruses are reported to be serious in the banana growing tract of India (Singh, 2002) and development of quick and reliable detection techniques for these banana viruses is a pre-requisite. The international standard for banana indexing is through ELISA (Diekmann and Putter, 1996) and this serological methodology is effective, although it is time consuming and costly.

Among the viral diseases, banana streak is a recently described virus which occurs in most of the banana producing countries. Hence, in the present project attempts were made to study the symptomatology, transmission and molecular diagnosis of the banana streak virus disease.

The development of symptoms of BSV was investigated in detail. Initially the symptoms appeared as discontinuous chlorotic streaks and later formed spindle shaped lesions. These chlorotic spindle shaped streaks form necrotic streaks and resulted in distortion of leaf lamina. Some cultivars showed symptoms of heart-rot and internal pseudostem necrosis. Such symptoms were earlier described as banana mosaic diseases (Wardlaw, 1972) which were later confirmed as banana streak disease. Likewise symptoms induced by BSV infection may sometimes resemble those caused by cucumber mosaic virus (CMV) and that

some of the earlier reports of BSV infection in banana incorrectly attributed to CMV rather than BSV (Singh, 2002)

The symptoms associated with the disease included leaf bases falling away from the pseudostem, narrow black streaks on the pseudostem, thicker leaves, cigar leaf necrosis and aberrant bunch emergence, reduced bunch size and distortion of fingers (Lassoudiere, 1974; Gauhl and Gauhl, 1994). Daniells *et al.* (1998) reported broad yellow lines on the leaf lamina parallel to the midrib, a purple margin to the leaf lamina and leaf twisting grooves in the base of the pseudostem in Williams (AAA, Cavendish Subgroup). Singh (2002) reported peel splitting, necrotic streaks and spot symptoms on fingers of Grand Nain (AAA, Cavendish Sub group). This type of symptom was not recorded in the present study. The symptom expression recorded in this study was similar to the symptoms reported from other tropical countries (Dahal *et al.*, 1998; Dahal *et al.*, 2000).

Lockhart (1986) observed an erratic distribution of symptoms on individual leaves, as well as on different leaves of the same plant and this feature is reported to be characteristic of BSV. In this study there was variation in the symptom expression on the individual plants as well as with changes in season. Symptom expression on some virus-host systems has been attributed to differences in the micro-climate of individual plants, or the differences in factors such as number of available infection sites, rate of virus multiplication and movement and virus concentration required for symptom expression (Scott and Rosencrantz, 1987).

In the present study, there was a significant negative relationship between BSV disease incidence and temperature. Dahal *et al.* (1997, 1998) demonstrated that temperature was an important factor for symptom expression in BSV-infected plants and relative virus concentration in the tissues. Under high temperature, BSV particles may be destroyed or their movement is restricted within the plant. Dahal *et al.* (1998) reported that in the case of BSV, the other possibility may be

that during dry and warmer season or at higher temperature BSV probably survives in the meristem shoots but does not replicate and during the rainy and cooler seasons, the virus particles may spread to the shoots leading to symptom expression. The reduction in symptom expression and severity under higher temperature conditions is also related to lower concentration of BSV virions.

In general, changes in morphological and biometric characters like height, girth and number of leaves of the plant may lead to change in the yield of that plant. In the present investigation on the effect of the disease on the yield parameters and fruit characters was assessed. Yield loss due to BSV infection in banana has been reported in AAA cultivar 'Poyo' in Ivory Coast by Lassoudiere (1974). Lockhart (1986) also reported reduction in bunch weight and malformation of fingers in Dwarf Cavendish (AAA). Lockhart and Olszewski (1993) reported the effect of BSV infection on plant growth, bunch yield and fruit quality may be variable. It has been suggested that BSV infection might have a significant effect on bunch size and fruit quality when floral initiation and early bunch development coincide with a period of increased virus synthesis. Dahal *et al.* (2000) reported that BSV-infected plants generally had significantly lower bunch weight, fewer hands and fewer fruits than apparently healthy plants.

As the studies on virus-vector relationship are necessary for the better understanding of the virus, the first approach was on the basic aspects of transmission. In the present study, the mechanical transmission, soil transmission and insect transmission were conducted and transmission of BSV could be achieved through mealy bug vectors. This is the first successful attempt of the transmission of BSV by *Dysmicoccus brevipes* Cockerell. Several aphid species such as *Aphis gossypii* Glover and *Rhopalosiphum padi* L. and *Pentalonia nigronervosa* Coquerel which briefly colonize banana failed to transmit BSV (Lockhart, 1986; Kubiriba *et al.*, 2001). Walkey (1991) reported that BSV is restricted to the phloem cells. The aphids may not possess the receptor cells for attachment of BSV, which may be present in the mealy bugs.

The results of the insect transmission using two groups of mealy bugs viz., *Dysmicoccus brevipes* Cockerell and *Ferrisia virgata* Cockerell found that both the mealy bugs act as vectors of BSV. Kubiriba *et al.* (2001) reported that the transmission rate of BSV was directly correlated with number of viruliferous mealy bugs *Dysmicoccus brevipes* Cockerell and is inversely correlated with age of the plant.

It was found that a single nymph or adult was not sufficient for the transmission of virus, but a minimum of 30 nymphs were required for the successful transmission of BSV. The explanation for this is that every vector exposed to the source may not necessarily be infective. Kubiriba *et al.* (2001) observed that the nymphs of mealy bugs are more efficient vectors of BSV than adult mealy bugs. The field transmission of BSV could be readily accomplished by early mealy bug instars when they were crawling between adjacent plants or were carried by wind to neighbouring plants. Also the rate of symptom expression was high during the rainy season and low in hot seasons.

Attempts on the effect of pre-acquisition fasting of nymphs and adults of *Dysmicoccus brevipes* Cockerell *and Ferrisia virgata* Cockerell revealed that fasting had an effect on the transmission of the virus. Moreover, the transmission rate was not reduced considerably as the pre-acquisition fasting was increased. Walkey (1991) reported that only semi-persistent relationship demands the pre-acquisition fasting for effective transmission.

In the present study on acquisition access period, it was found that nymphs and adults required minimum three days to acquire the virus from the source plant in the case of *Dysmicoccus brevipes* Cockerell and *Ferrisia virgata* Cockerell. Walkey (1991) reported that the optimum acquisition period of three days was for mealy bug vectors. Recipient plants inoculated by *Dysmicoccus brevipes* Cockerell tested positive four weeks after inoculation and in the case of *Ferrisia*

*virgata* Cockerell, it was six weeks. Kubiriba *et al.* (2001) reported that BSV was first detected in the pineapple mealy bug a day after transfer to virus sources. The proportion of mealy bugs carrying BSV increased to a peak on the third day after releasing it to BSV infected plant but thereafter declined.

In the experiment on inoculation threshold the minimum period required to transmit the virus to healthy plant was seven hours in the case of both nymphs and adults after an acquisition access of three days. Percentage of transmission increased with increase in the inoculation period. Kubiriba *et al.* (2001) reported that the minimum inoculation access period for transmission of BSV was seven hours. As the number of mealy bugs for transmission of virus is an important aspect in the virus-vector relationships, a minimum of 30 mealy bugs were needed for the transmission of BSV.

In the present investigation as the role of *Geococcus* sp. in the transmission of BSV, it was found that *Geococcus* sp. could not transmit BSV, though this mealybug was reported recently as a serious pest in the banana orchards of Kerala (Smitha *et al.*, 2005). Investigations are needed in future in this direction.

Transmission studies conducted through the planting materials indicated that infected suckers are the primary source of inoculum for BSV. Mechanical transmission conducted on hosts like colocasia, ash gourd, snake gourd, pumpkin, bitter gourd, bottle gourd and cowpea with different buffers at different pH revealed that BSV was not mechanically transmissible. The failure to infect banana by crude sap could be attributed to considerable amounts of latex and phenolic compounds and other inhibitory substances 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; Berg, 1962; Dale 1987; and Wu and Su, 1990). Lockhart (1986) reported that BSV was not transmitted mechanically from infected banana to healthy banana or to any of the other test plants. This also indicates that the spread of BSV through cutting tools

or during any cultural operation is will not occur. Soil transmission was not observed in the case of BSV. Lockhart (1986) also reported that BSV is not soil borne. The experiment on sucker transmission revealed that BSV could be transmitted through suckers. This result is in conformity with that of Daniells *et al.* (2001), who reported that banana streak disease is transmitted through infected suckers up to 100 per cent.

Badna viruses are the only second group of double stranded DNA plant viruses to be described(Lockhart and Olszweski,1994).The virions of BSV are non-enveloped, bacilliform with an average size of 150nm x 30nm and contain a circular double stranded DNA genome of size 7.5kb (Medberry *et al., 1990*; Lockhart and Olszewski, 1993). The electron microscopic observation conducted with the infected samples of banana revealed the presence of 130-150nm x 30nm sized bacilliform particles, thus confirming that the disease is caused by BSV. According to the latest ICTV (International Committee on Taxonomy of Viruses), this virus belongs to the genus Badnavirus and family Caulimoviridae (Van Regenmortal, 2000).

In the present study, the field gene bank maintained at BRS, Kannara was screened for the natural occurrence of the disease. Out of 290 accessions, seven were found to be infected. The Percent disease incidence, Percent disease severity, Coefficient of infection and Symptom severity index were calculated. The infected varieties were Mysore poovan(AAB), Motta poovan(AAB), Kalibale(AAB), Chandrabale (AAB), Nendran (AAB), Chinali (AAB), and FHIA-3 (AAAB). Sebasigari and Stover (1988) reported that Banana streak disease was recorded in Kabungo, 'Ney Poovan; (AB), 'Pisang Awak' (AAB) and the East African high-land beer-making cultivars of the 'Lujugira-Mutika' subgroup (AAA). In the Bukoba district of Tanzania, the disease was seen on 'Gros Michel' (AAA) and cooking cultivars in the 'Lujugira-Mutika' (AAA) subgroup. They also reported the incidence of the disease on Giant Cavendish (AAA), Cavendish subgroup and 'Pukusa' [(AAB), syn.Silk]. Later Dabek and Waller (1990) recognized Banana streak

symptoms in Mjenga, 'Kipaka' (AA, syn. Green Red), Mkono Wa Tembo (AAB, Horn plantain type), 'Kikonde Kenya' (AAB, syn. Mysore) and 'Kijakaze' (AAB, Pome Subgroup) in the germplasm collection in Zanzibar. In India, this disease was first confirmed on banana cv. Poovan (AAB) (Thangavelu and Singh., 1996). The occurrence of the disease on Mysorepoovan (AAB) and *Musa* hybrids was also reported by Cherian *et al.* (2002). Now in India, the presence of BSV has been confirmed from other states such as Karnataka, Andhra Pradesh, West Bengal, Bihar, Assam, and Gujarat (Singh, 1996). As the future line of work, the reaction of all the accessions of the field gene bank could be assessed by artificial inoculation and confirming the infection by applying the PCR technique standardized in the study.

Reliable diagnosis of BSV in banana is complicated by several factors arising from the nature of the disease and of the causal agent itself. Firstly, diagnosis based on foliar symptoms may be very unreliable because of the sporadic nature of symptom expression through out the year (Jones and Lockhart, 1993). Symptoms may be totally absent, or may be indistinct, under certain conditions. Symptom expression might be masked in plants derived from *in vitro* multiplication. For many plant viruses, biological indexing using indicator plants represents a simple and inexpensive method of testing propagating materials for virus infection. Because BSV infects only *Musa* and *Ensete* and is not transmissible to these species by mechanical inoculation, this method of indexing cannot be used for BSV (Lockhart, 1995).

Enzyme linked immunosorbent assay (ELISA) indexing protocols, using polyclonal antisera raised against a mixture of BSV antigens, have been partially successful in screening banana for BSV infection. But the procedure is not very reliable since it failed to detect a number of isolates of the virus (Lockhart and Olszewski, 1994). Immunosorbent electron microscopy of viral mini preps, using a multi strain antiserum to BSV is considered as most reliable method for detection of BSV strains. But the limited facility of electron microscopy and complex virus purification and immunization step in the production of antibodies

makes immunosorbent electron microscopy less preferable for routine detection (Cherian *et al.*, 2004).Hence PCR based detection would be more useful.

Polymerase chain reaction is widely used as one of the most important inventions of the 20th century in molecular biology .Small amounts of the genetic material can now be amplified to identify and detect infectious organisms, including the viruses. Singh (2002) emphasized the importance of PCR as the most sensitive molecular technique to detect the viral diseases. PCR involves three steps namely denaturation, annealing and extension. First the genetic material is denatured, converting the double stranded DNA molecules to single strands. The primers are then annealed to complementary regions of the single stranded molecules. In the third step, they are extended by the action of the DNA polymerase.

With its relative simplicity and high sensitivity, the PCR method has high potential for detecting minute quantities of virus in plant tissues. Cherian *et al.* (2004) reported that PCR using primers from conserved domain of genomes of badna viruses proved to be very useful for rapid, sensitive and reliable detection of different badna viruses. Initially optimization of the PCR conditions was carried out, since the PCR parameters are very critical to amplify the DNA fragments with specific primers.

In this present study, attempts were made to standardize the molecular indexing of BSV using PCR. The molecular diagnosis of BSV using PCR polymerase chain reaction in the varieties Nendran (AAB) and Mysore poovan (AAB) was carried out using specific primers at an annealing temperature of 59° C and a template dilution of 1:10 along with primer dilution of 1:10. The approximate size of the expected band was 730 bp. This conforms with the work of Cherian *et al.* (2004) who reported the molecular cloning of BSV infecting banana.

Isolation of plant genomic DNA is a requirement for PCR, genome characterization, mapping and isolation of genes for molecular biological studies. A good extraction protocol should yield pure, intact and adequate DNA. In the present study, DNA was extracted from banana leaves showing characteristic symptoms of BSV in cultivars namely Mysorepoovan (AAB), Nendran (AAB) and from healthy control.

The concentration of agarose gel was an important factor for the separation of DNA fragments. In this study 0.8 per cent gel was used for DNA samples while one per cent gel was used for PCR analysis.

For the standardization of PCR conditions, various combinations of parameters were tried. The tested parameters were template concentration and annealing temperature. Annealing temperature is an important factor governing amplification in PCR. If it is very low, no specific amplification will take place spurious product may get amplified, due to non-specific amplification. If the annealing temperature is very high, annealing will not take place or too little product will be made. In the present study, when annealing temperatures ranging from 57°C to 61°C was tried, optimum amplification of the product was obtained at 59°C using the primer combination BSV5466 and BSV6196.

Template concentration was another parameter that was standardized. Optimum amplification was obtained with a dilution of 1/10.With a decrease in template concentration, a decrease in the concentration of amplified product was observed. The intensity of amplification was reduced with increase in the template dilutions. Template concentration is a major factor affecting PCR. Amplification varies with the type and concentration of template. During each cycle, the template gets multiplied in the range of $2^n$, where n is the number of cycles. Good quality template was found to be necessary for better amplification.

IC-PCR is a PCR technique based on antigen-antibody reaction. It is a more sensitive, reliable and rapid technique for diagnosing BSV infections of *Musa* from both *in vitro* propagated and field grown plants. In IC-PCR, DNA isolation step can be avoided. IC-PCR is suitable for the large scale screening of *Musa* for episomal BSV which is necessary for germplasm movement. This technique could be used for detecting different serotypes of BSV (Harper *et al.*, 1998; Le Provost *et al.*, 2006). In the present study IC-PCR of BSV infected samples were also standardized using specific primers at annealing temperature 59 °C.

**Future line of work:-**

Molecular diagnostic probes could be developed. Virus resistant transgenic plants could be developed. Molecular diagnostic probes could be developed for the accurate and sensitive detection of episomal as well as integrated BSV. The chance of incidence of BSV in intercropping of banana with pineapple could be studied. Ecofriendly management of banana streak disease.

# *Summary*

## 6. SUMMARY

The study entitled "Symptomatology and molecular diagnosis of Banana streak virus disease" was carried out at the Department of Plant Pathology and Banana Research Station, Kannara during 2008-2010.

The salient findings of the study are summarized below.

The naturally infected suckers collected from different fields of Kannara and the virus culture maintained at Banana Research Station, Kannara were used as the source of inoculum for the study. Symptomatology of BSV infected plants was studied on different varieties viz., Mysorepoovan (AAB), Mottapoovan (AAB), Kalibale (AAB), Nendran (AAB) and Chandrabale (AAB).The characteristic symptoms at each growth stages of the plant were documented. The symptoms expressed on different parts of the plant like leaves, petiole, pseudostem, male buds and bunches were also recorded. Major symptoms of BSV observed in the infected plants were discrete chlorotic streaks, spindle shaped chlorotic streaks, long streaks running parallel to lamina, changes to necrotic streaks on leaves. During hotter months these chlorotic or yellow flecks became necrotic and later leading to the distortion of leaf lamina. Necrotic or small dark/brown streaks were seen on the pseudostem of BSV infected varieties. Such lesions were also appeared on the petiole. BSV infected plants produced undersized bunches. Severely infected plants failed to flower was recorded in the case of Nendran (AAB) and Mysorepoovan (AAB).

Biometric characters were significantly influenced by the disease. Due to the disease, There was a reduction in the plant height, girth, number of leaves in diseased plants when compared to healthy plants. The disease adversely affected the yield characters.The bunch weight was drastically reduced in those varieties which flowered viz.,Kalibale (AAB) and Motta poovan(AAB).Severely infected plants of Nendran(AAB) and Mysore poovan(AAB) failed to produce any bunch.

The number of hands per bunch and number of fingers per bunch were also found to be reduced due to infection. The disease affected the length, girth and weight of finger in all varieties,this might be the reason for the reduction in the bunch weight.

The field gene bank of 290 accessions maintained at BRS, Kannara was screened at monthly intervals from planting till harvest for the natural occurrence of the disease. Among 290, accessions screened 283 were free of any characteristic symptom of BSV. Scoring of infected suckers was done based on 0-6 scale. Percent disease incidence, Percent disease severity, Coefficient of infection and Symptom severity index was calculated.

The mechanical transmission studies clearly indicated that BSV was not mechanically transmissible by using crude sap as well as by purified virus preparations. Soil transmission studies were conducted and the results indicated that BSV was not transmitted through soil. The sucker transmission studies showed that there was successful transmission of infection through suckers.

Insect transmission studies conducted with aphids and two species of mealy bugs- *Dysmicoccus brevipes* Cockerell and *Ferrisia virgata* Cockerell. Studies revealed that aphids are not the vectors of BSV. Both the mealy bugs successfully transmitted the virus to healthy plants. Virus –vector relationship studies revealed that BSV was transmitted in a semi-persistent manner by mealy bugs- *Dysmicoccus brevipes* Cockerell and *Ferrisia virgata* Cockerell. Adults and nymphs acted as efficient vectors in carrying the disease.

Electron microscopic investigation of the infected leaf samples was done to study the morphology of the BSV particles. The electron microscopic observations of infected leaf of banana showed typical bacilliform particles of size 130-150 × 30nm.

The molecular diagnosis of BSV using polymerase chain reaction on varieties Nendran (AAB) and Mysore poovan (AAB) was carried out using specific primers designed from the region corresponding to the conserved domain of reverse transcriptase and RNase H. PCR was carried out with the primers at an annealing temperature of 59° C and a template dilution of 1:10 along with primer dilutions of 1:10. The approximate size of the expected band was 730 bp. In this study IC-PCR of BSV infected samples were standardized using specific primers at same annealing temperature in which PCR was carried out.

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# Appendix

# Appendix

# APPENDIX I

**Composition of different buffers used in mechanical transmission**

**1. 0.1 M Sodium borate buffer (pH 8.0)**

A. Boric acid (0.2 M) - 1.237g/100ml

B. Borax $Na_2B_4O_7.10H_2O$ - 1.907g/100ml

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 - 6.084g/500ml

B. 0.1 M Dipotassium hydrogen phosphate - 8.079g/500ml

0.1 M (pH 7.2) – 28ml of A mixed with 72 ml of B.

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

(0.01 M DIECA) – 0.225g/100ml

**3. 0.1 M Sodium phosphate buffer (pH 7.2)**

A. 0.1 M Sodium phosphate monobasic anhydrous - 5.999g/500ml

B. 0.1 M Sodium phosphate dibasic dehydrate - 8.899g/500ml

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 - 2.101g/100ml

B. 0.1 M Sodium citrate - 2.940g/100ml

1.6 ml of A mixed with 18.4 ml of B

**5. 0.1M Tris buffer (pH 7.2)**

Tris - 24.23g/1000ml

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

# APPENDIX II

**Composition of buffers and dyes used in gel electrophoresis**

**1. 6X Loading/Tracking dye**

| | | |
|---|---|---|
| Bromophenol blue | - | 0.25% |
| Xylene cyanol | - | 0.25% |
| Glycerol | - | 30% |

The dye was prepared and stored at $4^0$ C.

**2. Ethidium Bromide (intercalating dye)**

The dye was prepared as a stock solution of 10 mg/ml in water and was stored at room temperature in a dark bottle.

**3. 50X TAE buffer (pH 8.0)**

| | | |
|---|---|---|
| Tris base | - | 242.0 g |
| Glacial acetic acid | - | 57.1 ml |
| 0.5M EDTA (pH 8.0) | - | 100ml |
| Distilled water | - | 1000ml |

The solutions was prepared and stored at room temperature.

# APPENDIX III

**Weather parameters of the experiment site in the year 2008**

| Year-2008 | Temperature | | Relative | Rainfall | Sunshine |
|---|---|---|---|---|---|
| Months | Max | Mini | Humidity | | hours |
| January | 32.3 | 21.7 | 59 | 0 | 292.9 |
| February | 33.6 | 2.9 | 61 | 29.7 | 236.9 |
| March | 33.2 | 23.4 | 64 | 205.3 | 212.5 |
| April | 33.2 | 24.9 | 75 | 65.6 | 189.9 |
| May | 33.0 | 24.7 | 73 | 11.5 | 188.6 |
| June | 29.9 | 23.5 | 85 | 636.7 | 59.0 |
| July | 29.3 | 23.2 | 84 | 416.3 | 84.9 |
| August | 29.8 | 23.6 | 82 | 321.9 | 106.5 |
| September | 30.6 | 23.2 | 80 | 314.2 | 160.7 |
| October | 31.7 | 23.4 | 76 | 380.8 | 176.2 |
| November | 32.2 | 23.1 | 70 | 21.7 | 180.3 |
| December | 31.6 | 22.5 | 60 | 2.6 | 238.9 |

**Weather parameters of the experiment site in the year 2009**

| Year-2009 | Temperature | | Relative Humidity | Rainfall | Sunshine hours |
|---|---|---|---|---|---|
| Months | Max | Mini | | | |
| January | 32.8 | 21.9 | 54 | 0 | 290.0 |
| February | 35.1 | 22.1 | 57 | 0 | 277.2 |
| March | 35.1 | 24.4 | 70 | 29.0 | 245.2 |
| April | 34.1 | 25.3 | 74 | 16.5 | 173.2 |
| May | 33.0 | 24.8 | 77 | 199.5 | 170.1 |
| June | 30.0 | 23.7 | 84 | 565.0 | 116.2 |
| July | 28.6 | 22.8 | 88 | 985.8 | 53.1 |
| August | 30.2 | 23.2 | 85 | 421.4 | 125.7 |
| September | 30.0 | 23.2 | 83 | 276 | 120 |
| October | 32.0 | 23.2 | 77 | 166.8 | 209.1 |
| November | 31.5 | 23.7 | 76 | 180.6 | 171.4 |
| December | 31.8 | 23.9 | 62 | 42.7 | 241.6 |

**Weather parameters of the experiment site in the year 2010**

| Year-2010 | Temperature(°C) | | Relative Humidity (%) | Rainfall (mm) | Sunshine hours |
|---|---|---|---|---|---|
| Months | Max (°C) | Mini (°C) | | | |
| January | 32.5 | 22.7 | 61 | 0 | 280 |
| February | 34.9 | 23.7 | 59 | 0 | 253.6 |
| March | 36.2 | 24.8 | 65 | 12.9 | 258.9 |
| April | 35.1 | 25.2 | 73 | 103.6 | 221.7 |
| May | 33.1 | 25.6 | 79 | 123.8 | 166.5 |

# APPENDIX IV

**Composition of buffers used in IC-PCR**

**1. 1X Phosphate buffered saline (PBS) pH 7.4**

| | | |
|---|---|---|
| 137mM NaCl | - | 8g/1000ml |
| 1.4mM $KH_2PO_4$ | - | 0.2g/1000ml |
| 8mM $Na_2HPO_4$ | - | 1.16g/1000ml |
| 2.6mM KCl | - | 0.2g/1000ml |
| Distilled Water | - | 1000ml |

**2. Wash buffer,PBS-Tween (PBS-T)**

To 1 litre of 1X PBS, add 0.5ml Tween.

**3. Coating buffer (CB) pH 9.2**

| | | |
|---|---|---|
| 15mM $Na_2CO_3$ | - | 1.59g/1000ml |
| 35mM $NaHCO_3$ | - | 2.93g/1000ml |
| 3.2mM $NaNO_3$ | - | 0.28g/1000ml |
| Distilled water | - | 1000ml |

# Abstract

# SYMPTOMATOLOGY AND MOLECULAR DIAGNOSIS OF BANANA STREAK VIRUS DISEASE

By

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**( 2007 - 11 - 106 )**

## ABSTRACT OF THE THESIS

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## ABSTRACT

The banana (*Musa* spp.) is a crop of global importance in terms of income security to million of small farmers throughout the developing countries. It is the world's fourth most important commodity after rice, wheat and corn and is produced in tropical and subtropical regions. Banana is infected by several diseases caused by fungi, bacteria and viruses. Among the viral diseases, Banana streak is now emerging as a major disease affecting banana production world wide. This disease assumes significance as it affects plant growth, fruit yield and quality. It is also causing problems to germplasm exchange and in the certification of *in vitro* plantlets for international trade.

The present project was undertaken to study the symptomatology of Banana streak disease, to investigate the role of root mealy bug - *Geococcus* sp. in the transmission of Banana streak virus, to standardize molecular indexing of planting materials of banana and to identify the source of resistance in the field gene bank.

The symptoms of the disease appeared on different parts of the plant such as leaf lamina, midrib, pseudostem and in bunches. On the leaf lamina, the symptoms developed as discontinuous or continuous linear small chlorotic streaks. These chlorotic streaks later turned necrotic, blackened and running perpendicular to the leaf axis extending from midrib to the leaf margin or sometimes form a linear mosaic like pattern on the lamina especially on older leaves. Dark brown coloured linear lesions appeared on other parts like petiole, midrib, pseudostem and on bunches. Under severe conditions, necrosis and death of cigar leaf was noticed. The plants showing such symptoms did not flower and resulted in 100 percent yield loss. The impact of the disease on biometric and yield characters was studied and observed that the disease affected the growth and yield of banana. A significant correlation was observed between the expression of symptoms with rainfall and temperature. The expression of the symptoms was more in cooler months and less in summer.

The field gene bank comprising 290 accessions maintained at BRS, Kannara was screened to assess the reaction of these accessions to the disease. The disease incidence

was recorded on seven accessions viz., Mottapoovan (AAB), Mysorepoovan (AAB), Kalibale (AAB), Chandrabale (AAB), Chinali (AAB), Nendran (AAB) and FHIA-3 (AAAB). The percent disease incidence ranged from13.25 to 32.16 .

The transmission studies proved that BSV was not transmitted mechanically or through infected soil. The insect vectors of BSV were proved to be two species of mealy bugs such as *Dysmicoccus brevipes* (Cockerell) and *Ferrisia virgata* (Cockerell). The studies on virus vector relationship of these mealy bugs showed that the maximum acquisition feeding period, pre-acquisition fasting period, inoculation access period required for successful transmission were three days, one hours and seven hours respectively. The nymphs were more efficient vectors than adults. A minimum of thirty numbers were required for successful transmission of BSV. Plants inoculated with *Dysmicoccus brevipes* (Cockerell) produced symptoms four weeks after inoculation and in the case of *Ferrisia virgata* (Cockerell), it was six weeks.

Recently, the root mealy bug - *Geococcus* sp. is becoming a serious pest in banana orchards of Kerala. Hence studies were conducted to investigate whether this mealy bug has any role in the transmission of BSV. It was found that *Geococcus* sp. could not transmit BSV. The banana aphid - *Pentalonia nigronervosa* Coquerel,the vector of Banana bunchy top disease had no role in the transmission of the virus. The studies on the transmission of the BSV through planting material proved that BSV is naturally transmitted through the planting materials of banana.

PCR based molecular diagnosis is one of the reliable and quick method for the virus indexing of planting materials. The molecular diagnosis of BSV using polymerase chain reaction from infected samples was standardized using specific primers, (BSV 5466-5'AGAGTGGGTTTCATCAAGTAGC and BSV 6196-5' GAATTTCCCGCTCGCATAAG) at an annealing temperature of 59° C. Immunocapture polymerase chain reaction (IC-PCR) of BSV infected samples was also standardized using the antiserum of BSV By IC-PCR, the detection of episomal virus infection could be done directly from the crude sap, avoiding the step of DNA isolation.

The outcome of this study will facilitate early detection and elimination of BSV infected plants and ensure distribution of healthy planting materials both suckers and tissue culture plants to the farmers of Kerala. Thereby, increasing the production as well as the productivity of banana in the state.