



A MINI REVIEW ON "BRACT MOSAIC" DISEASE OF BANANA

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ABSTRACT

The disease "bract mosaic" in banana and plantain was caused by Banana bract mosaic virus (BBrMV), a distinct member of the genus Potyvirus belongs to the family Potyviridae. The disease incidence was reported from various parts of the world including Philippine, Australia, Sri Lanka, India, Western Samoa and Vietnam. In India, the disease is more prevalent in states of Kerala, Karnataka, Tamil Nadu (TN) and Andhra Pradesh (AP). BBrMV is a non-enveloped flexuous filamentous ssRNA virus infects all kinds of banana varieties. It follows the putative polyprotein strategy of Potyvirus. The virus is transmitted by aphid (non-persistently) and vegetative planting materials and account for significant yield loss in banana. Recently, in addition to banana, the virus was also reported in small cardamom (*Elettaria cardamomum*) and flowering ginger (*Alphinia purpurata*) indicates adapting new hosts for their surveillance and spread. A laboratory study demonstrated that BBrMV could be transmitted from flowering ginger to its natural host banana seems alarming sign to the global banana production. The major problem in the management of this disease is remains unnoticed until it causes severe crop loss. Regular field inspection, early disease diagnosis, removal of source of virus inoculums, insect vector control, selection of resistant plants, mass production virus-free banana seedlings by in vitro micro-propagation and stringent quarantine measures are necessary for better management of the disease. This review provides an overview of distribution, properties, detection and management of BBrMV.

KEY WORDS

Banana bract mosaic virus, banana, disease incidence, sequence, detection.

INTRODUCTION

Banana and Plantain (hereinafter referred to as banana) belongs to the genus *Musa*, family *Musaceae*, the fourth most important food crop in the world after rice, maize and wheat. India is the largest banana producing country (29.1 million tonnes) in the world [1]. Banana production was severely threatened by many microbial pathogens including fungi, bacteria and viruses. At present about 18 viruses were identified in banana across the world and their infections severely affected the agronomy and economics of banana. Of the 18 viruses viz., "Banana bunchy top virus (BBTV, a *Babuvirus*), Banana streak Mysore virus (BSMYV, a *Badnavirus*), Banana streak IM virus (BSIMV, a *Badnavirus*), Banana streak UA virus (BSUAV, a *Badnavirus*), Banana streak UI virus (BSUIV, a

Badnavirus), Banana streak UL virus (BSULV, a *Badnavirus*), Banana streak UM virus (BSUMV, a *Badnavirus*), Banana streak VN virus (BSVNV, a *Badnavirus*) Banana streak Gold finger virus (BSGFV, a *Badnavirus*), Banana streak Obino l'Ewai virus (BSOLV, a *Badnavirus*), Banana bract mosaic virus (BBrMV, a *Potyvirus*), Banana mild mosaic virus (BanMMV, *Betaflexiviridae*), Banana virus X (BVX, *Betaflexiviridae*), Cucumber mosaic virus (CMV, a *Cucumovirus*), Abaca mosaic virus [AbaMV, a distinct strain of *Sugarcane mosaic virus* (SCMV) designated as SCMV-Ab, a *Potyvirus*], Abaca bunchy top virus (ABTV, *Babuvirus*), Tobacco mosaic virus (TMV, a *Tobamovirus*) and Banana die-back virus (BDBV, a probable *Nepovirus*)" of banana [2], BSV (majorly BSMYV), BBTV,

BBrM and CMV were widely distributed in India and account for significant yield loss.

BBrMV is the causal agent of "bract mosaic" disease in banana. Yield loss of up to 40% was recorded in highly susceptible varieties particularly in ABB cooking banana cultivar 'Saba' and 'Cardaba' [3,4]. The disease was first reported in Philippine island of Mindanao in 1979. Later the disease was found to be widespread in several countries including Australia, Sri Lanka, India, Western Samoa and Vietnam [5]. The virus is known to infect mainly *Musa* spp. however, recently identified its natural infection in small cardamom (*Elettaria cardamomum*) and flowering ginger, (*Alphinia purpurata*) [6,7].

In India, a disease locally called "Kokkan" with unknown etiology was recognized on Nendran variety (AAB, sub group) in Kerala in 1966, which was now confirmed as bract mosaic disease [8,9]. Subsequently, the typical bract mosaic symptoms on banana cultivars were noticed in 1992 in the germplasm collections at Indian Institute of Horticultural Research (IIHR) and Tamil Nadu Agricultural University (TAU) in Bangalore and Coimbatore, respectively [5]. Characteristic symptoms for bract mosaic disease were found on banana in West

Godavari district (Andhra Pradesh) and the causal virus was identified as BBrMV [10]. Further, banana samples suspected for bract mosaic disease from Tamil Nadu and Maharashtra states were found to contain potyvirus-like particles in leaf dip preparations [11, 12]. The virus poses a considerable quarantine risk due to its limited distribution, and its ability to rapidly spread through vegetative propagules (suckers) or aphids [13].

SYMPTOMS AND CYTOPATHOLOGY

The infected banana plants show distinct symptoms on various parts of the plants. The characteristic symptoms are dark red-brown mosaic patterns on flower bracts, reddish-brown spindle like streaks on pseudostem, green or red streaks or spindle-shaped lesions on leaf petioles and midribs of younger leaves and chlorotic streaks on the leaves [14,9,15] (Fig 1). Splitting of the leaf sheath along the discolored region was commonly observed and in severe infection leads to fruit rejection [16,14]. Cytoplasmic pinwheel inclusions and scrolls were observed in infected cells of leaf sample of banana [14]. Appearance of these inclusions, the main feature of potyviruses.

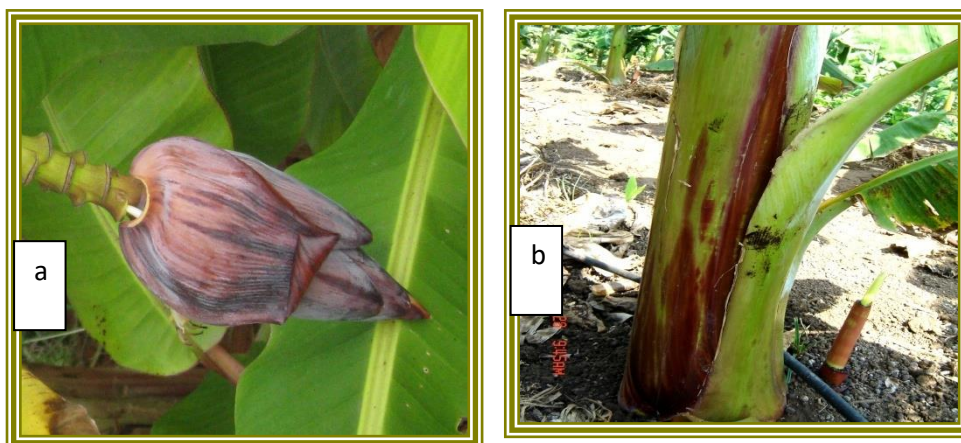


Fig 1. a) Characteristic dark red-brown mosaic patterns on flower bracts, (b) reddish-brown spindle like streaks on pseudostem.

TRANSMISSION AND HOST RANGE

There were at least three species of aphids including "*Pentalonia nigronervosa*, *Rhopalosiphum maidis* and *Aphis gossypii*" involved in transmission of BBrMV in a non-persistent manner [13,17,18]. The virus can be transmitted through vegetative planting material including suckers, bits and corms, and via micro-propagated plantlets of banana [5]. The virus is known

to infect mainly banana, however, recently reported in small cardamom (*Elettaria cardamomum*) and flowering ginger (*Alphinia purpurata*), respectively in India and Hawaii [6,7]. Attempts to transmit BBrMV by sap inoculation to herbaceous indicator plants were unsuccessful [13,19] (Magnaye and Espino, 1990; Diekmann and Putter, 1996). A laboratory study demonstrated that BBrMV could be transmitted from flowering ginger to its natural host banana [20].

Table 1. Known functions of potyviral proteins [29-32].

Protein	Functions
P1 (32-64 kDa)	Serine proteinase with <i>cis</i> -cleavage activity, Accessory factor for virus amplification, Host adaptation, P1/HCpro cleavage site, Responsible for symptomatology, Usable for investigation of potyvirus phylogeny and relationship.
HC-Pro (56-58 kDa)	Cysteine proteinase with <i>cis</i> -cleavage activity, HCpro/P3 cleavage site, Helper factor for aphid transmission, RNA silencing suppression, Viral replication and packaging, Enhancement of yield of virus particle, Systemic movement, Symptoms development.
P3 (37 kDa) P3N-PIPO (~7kDa) 6K1	Virus amplification, Host adaptation. Cell-to-cell movement. Cell-to-cell movement? Modulation of P3 activity? Role in virulence?
CI (70 kDa)	RNA helicase, RNA replication, Virus movement, Pinwheel inclusions formation, Development of symptoms, Breaking the host resistance.
6K2	Membrane vesicles proliferation, Membrane targeting, Long distance movement, Development of systemic infection.
VPg	Covalently links the 5' end of the viral RNA via tyrosine residue, Primer of RNA replication, RNA translation, Virus movement, Systemic infection, Overcoming the eIF4E-based recessive resistance.
NIa/Pro	Cystein protease, Host specificity, Host DNA cleavage activity.
Nib CP (11-20 nm in dia, 680-900 nm in length,38-39kDa)	RNA dependent RNA polymerase. Protection of genomic RNA, Virus movement, Aphid transmission, Seed transmission, Virus replication.

GENOME ORGANIZATION

BBrMV has non-enveloped flexuous filamentous virions, each 750 nm x 11 nm [14]. The purified virions contain a major coat protein (CP) of 38-39 kDa and buoyant density in caesium chloride of 1.29-1.31 gcm⁻³ and A_{260/280} of 1.17[14]. Recently, the complete genome sequence of BBrMV of banana was established [21,22]. The genome of BBrMV is a positive sense ssRNA that comprised 9711 nucleotides, excluding polyA tail but

including the 5' and 3' untranslated regions which comprised 128 and 208 nucleotides, respectively. As like other potyviruses, the 5' UTR of BBrMV is rich with A/T residues of 60% and contains two potybox-like blocks namely potybox a (TCAGCAAGACA) and potybox b (TTACGCA). The genome of BBrMV encodes a putative polyprotein of 3125 amino acid residues in length which subsequently cleaved into 10 proteins namely P1, HCpro, P3, 6K1, CI,6K2, VPg, NIa, NIB and CP with 329,

457, 347, 52, 634, 53, 190, 243, 520 and 300 amino acid residues, respectively (Fig 2, Table 1). In addition, a small P3N-PIPO protein derived from a separated small ORF was also observed in BBrMV [23], probably involve in the movement of virus. The first in-frame ATG codon of BBrMV genome is CAAATGG. Complete genome

sequence analysis of BBrMV isolates of *Elettaria cardamomum* and *Alphhinia purpurata* revealed that their genome organization also similar to the BBrMV of banana. The functions of the putative proteins of BBrMV as similar to other potyviruses (Table 1)

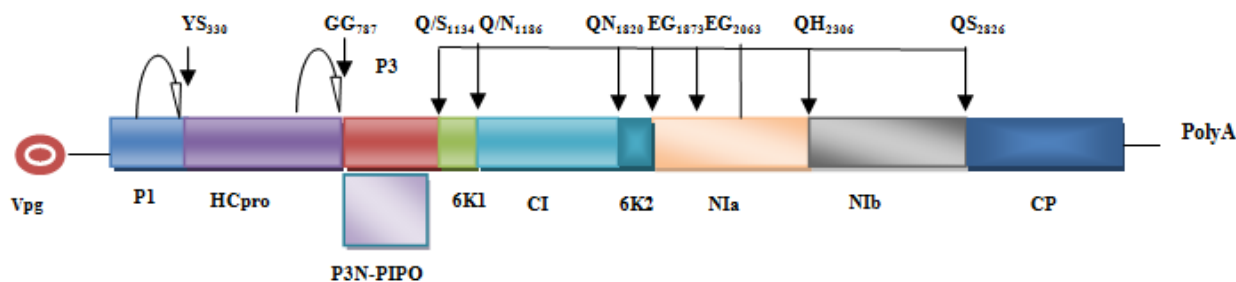


Fig 2. BBrMV genome map representing various putative protein regions with respective cleavage sites.

GENETIC DIVERSITY

It explains mainly the rate of variations at genome levels over a period of time in a population. These variations possible through the influence of various factors including biological and physical factors. In potyviruses, the CP cistron and the 3'UTR were used to discriminate viruses. Analysis of 136 possible pairings of complete CP amino acid sequences from 17 strains of eight potyvirus species revealed a bimodal distribution of sequence identity in which distinct potyvirus species exhibited 38-71% amino acid similarities, while strains shared 90-99% amino acid similarities [24]. In addition, Frenkel et al., [25] suggested that the nucleotide sequence of 3'-UTR of potyvirus strains was highly conserved (83-99%) where as distinct potyviruses had similarity of 30-53%. Pairwise comparisons made between all the available complete coat protein sequences (1220 sequences, 743590 comparisons) of the family *Potyviridae* showed 49-52% and 76-78% identity at amino acid and nucleotide levels, respectively [24]. The complete genome sequence of BBrMV-TRY was 94% identical with BBrMV-PHI isolate at the nucleotide level and its ten mature proteins had amino acid sequence identities ranging from 88 to 98%. Phylogenetic analysis suggests that the BBrMV-TRY isolate is closely related to the BBrMV-PHI isolate [22]. Multiple alignments of CP gene of 49 BBrMV isolates showed nucleotide (nt) and amino acid (aa) identity of 79-100% and 80-100 %, respectively. Phylogenetic analysis revealed that except two Indians isolates (TN14 and TN16), all isolates clustered together [23].

DETECTION

Symptom based disease diagnosis is always not worthwhile in detecting BBrMV in banana. Depending on the cultivar and conditions existed, BBrMV may or may not express its characteristic symptoms on infected banana plants. Sometimes the leaf symptoms of BBrMV are similar to that of symptoms caused by CMV or BSV in *Musa spp.* It misleads accurate detection of virus. Various formats of sero and molecular diagnostics were developed to detect BBrMV in banana. Thomas et al., [14] raised polyclonal antibodies (PABs) against purified virions of a Philippine isolate of BBrMV from field-infected banana cv. Cardaba. Further, Rodoni et al., [11] expressed the entire CP region of BBrMV (P1 isolate) in *E.coli* and the purified recombinant coat protein (rCP) used as an antigen for production of polyclonal antibodies. Agdia Inc (USA), commercially providing monoclonal antibodies (MAbs) to BBrMV. All these antibodies were used in ELISA, Dot-ELISA, immunoblotting and immune capture PCR (IC PCR) to detect BBrMV in banana.

The genome-based detection techniques like RT-PCR, IC-RT-PCR, northern blot hybridization have been adopted to detect BBrMV in banana. Virus (BBrMV) specific cDNA was amplified from field samples by RT-PCR using potyvirus degenerate primers [14]. Rodoni et al., [9] have confirmed the presence of BBrMV in India by RT-PCR in plants showing symptoms similar to those caused by CMV. DIG labeled DNA probes for BBrMV were developed and used in northern blot hybridization for the detection of BBrMV in infected banana plants [14,9]. Rodoni et al., [11] developed IC-RT-PCR for

BBrMV detection in banana. A multiplex-PCR technique was developed to detect BBrMV along with other viruses infecting banana [26]. One-step RT-PCR assay is sensitive, specific for the detection of a broad range of BBrMV isolates and suitable to quarantine and gemplasm detections [27].

MANAGEMENT AND CONTROL

Management of bract mosaic disease is quite difficult in banana. The disease remains unnoticed until it causes severe damage to the crop. Selection of virus resistant plants, production of virus free seedlings through *in vitro* micro-propagation, elimination of source of virus inoculums in the field are the best options for efficient control of bract mosaic disease. However, so far, no resistant banana cultivar is successfully developed for BBrMV. Recently, putative resistant line of Abaca (*Musa textilis* Nee) cvs. viz., Tinawagan Pula (TP) and Tangongon (TG) were developed to BBrMV through *in vitro* mutagenesis by exposing gamma irradiation showed significant disease resistance [28]. However, these promising lines are yet to be evaluated for other horticultural traits at field level. To produce virus free banana seedlings through tissue culture, the initial planting material must be indexed for virus by efficient and reliable diagnostic methods. Serological and molecular diagnostics were adopted to detect BBrMV in banana. Various formats of ELISA (DAC ELISA, Dot-ELISA and TAS-ELISA) and PCR methods (RT-PCR, IC RT-PCR, One-step RT-PCR) were in use to detect bract mosaic disease in banana. Rouging disease indexed banana plants is an important measure for field sanitation. Rouging/sanitation programmes have introduced into commercial production areas in Philippines (Magnaye, 1994). The stringent quarantine and certification measures prevent the spread of the disease into new geographical regions.

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