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Occurrence and distribution of banana streak disease and standardization of a reliable detection procedure for routine indexing of banana streak viruses in India

Susheel Kumar Sharma[a,1], P. Vignesh Kumar[a], Rajshri Poswal[a], Richa Rai[a], A. Swapna Geetanjali[a,2], K. Prabha[a,b], Rakesh Kumar Jain[a], Virendra Kumar Baranwal[a,*]

[a] Advanced Centre for Plant Virology, Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi 110012, India
[b] Directorate of Floricultural Research, MPKV College of Agriculture, Pune 411005, India

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ABSTRACT

In a survey for banana streak disease during 2010–2013, occurrence of typical streak symptoms was recorded with an incidence of 20–50% in different banana orchards at different locations in India. Serological and molecular studies provided evidence for the presence of episomal *Banana streak MY virus* (BSMYV) and *Banana streak OL virus* (BSOLV) in the field samples collected from different banana growing regions. Various methods of virus detection for banana streak viruses (BSVs) were employed. Direct PCR and direct binding-PCR (DB-PCR) did not give accurate and confirmatory results for the presence of episomal virus infection. Rolling circle amplification (RCA) was found to be highly reliable but was time consuming and difficult to use for indexing of a large number of samples. The duplex-immunocapture-PCR (D-IC-PCR) employing polyclonal antiserum with an immunocapture time of 3 h was found to be a sensitive, reliable and accurate procedure for detection of episomal BSV infection. For definite detection of BSMYV, the widely occurring banana streak virus species in India D-IC-PCR using BSMYV specific primers and *Musa* specific internal primers was found to be most reliable procedure which can be used for routine indexing of tissue culture plantlets at different stages in the test labs as well as for germplasm exchange at quarantine stations. Based on the immuno- and immuno-nucleo detection procedures up to 46% of samples collected from different banana growing regions of India were found positive for BSV infection. Present study for the first time reported the widespread occurrence and distribution of BSV in India.

1. Introduction

Streak disease of banana is a major threat to banana production and improvement worldwide. The disease is caused by a cryptic virus species complex known as banana streak viruses (BSVs) (Dahal et al., 1998b; Hull et al., 2000; James et al., 2011b). At least eleven serologically and genetically heterogeneous virus species have been associated with the variable symptoms of chlorotic and necrotic streaking on leaf lamina, leaf distortion, stem cracking, abnormal bunch development, death of growing point and pseudostem cracking (Dahal et al., 2000). The disease has been reported from most of the banana growing regions of world including India (Cherian et al., 2004; Geering et al., 2005; Baranwal et al., 2014) and causes a significant yield reductions ranging from 7% to 90% (Lockhart, 1986).

BSVs are mealybug transmitted plant pararetroviruses in the genus *Badnavirus* under the family *Caulimoviridae* having non-enveloped bacilliform virus particles of 30 × 130–150 nm with a double stranded circular DNA genome of 6.9–7.8 kb which is non-covalently closed (King et al., 2011). BSV genome encodes three open reading frames (ORFs); ORF1 (unknown function), ORF2 (virion associated protein) and ORF3 a multifunctional polyprotein which is cleaved to movement protein (MP), coat protein (CP), aspartic protease (AP), reverse transcriptase (RT) and ribonuclease H (RNase H) after translation (King et al., 2011).

A very peculiar characteristic is the presence of host integrated BSV like sequences in the banana genome known as endogenous BSV (eBSV). These eBSV sequences become a mean of vertical

* Corresponding author. Tel.: +91 11 2584 3113; fax: +91 11 2584 3113.
E-mail addresses: vbaranwal2001@yahoo.com, vbaranwal2001@gmail.com (V.K. Baranwal).
[1] Present address: ICAR Research Complex for NEH Region, Manipur Centre, Lamphelpat, Imphal 795004, India.
[2] Present address: Department of Genetic Engineering, SRM University, Chennai, Tamil Nadu 603203, India.

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transmission of BSV, leading to episomal BSV infection which originated from the excision of eBSV in *Musa balbisiana* genome (Gayral et al., 2008; Gayral and Iskra-Caruana, 2009). The activable eBSV sequences are known only in *M. balbisiana* (B) genome, whereas the *Musa acuminata* (A) genome contains the eBSV sequences which are known to be non-activable (Gayral and Iskra-Caruana, 2009). The eBSV counterparts for four episomal badnaviruses; *Banana streak OL virus* (BSOLV), *Banana streak MY virus* (BSMYV), *Banana streak GF virus* (BSGFV) and *Banana streak IM virus* (BSIMV) are currently known in the *Musa* genome (Harper et al., 1999b; Gayral et al., 2010; Chabannes et al., 2013). Stress such as micropropagation through tissue culture has been shown to activate the release of eBSV sequences in *Musa* genome into replicationally competent episomal viral infection (Dallot et al., 2001). In addition to tissue culture, other stresses such as temperature differences, water stress and genetic hybridization also activates the release of infectious virions (Dahal et al., 1998a; Lheureux et al., 2003). Thus the interspecific *M. acuminata* × *M. balbisiana* (A × B) hybrids usually shows the tendency to produce episomal BSV infection arising from integrated eBSV sequences (Gayral and Iskra-Caruana, 2009).

India is the largest producer of banana contributing to around 22% of total banana production worldwide (NHB, 2011). It is being grown in an area of 8.3 lakh ha with a total production of 297.8 lakh tonnes (NHB, 2011). Banana streak disease has been considered a major threat to tissue culture industry and breeding programmes in India. The management of banana streak disease largely relies on the availability of accurate, sensitive, low cost and robust diagnosis of BSV infection. Reliable, sensitive and accurate diagnosis of BSVs has been a major challenge worldwide. Different techniques like enzyme linked immunosorbent assay (ELISA) and polymerase chain reaction (PCR) has been developed for detection of BSV (Ndowora and Lockhart, 2000; Cherian et al., 2004; Sharma et al., 2014). PCR, due to the presence of integrated sequences leads to false positives (James et al., 2011a). ELISA although can be used to detect the presence of episomal virus infection usually gives erratic results due to the low virus titre in infected hosts. Recently, immunocapture PCR (IC-PCR) and rolling circle amplification (RCA) has been developed for the detection of episomal BSV infection (Le Provost et al., 2006; James et al., 2011a). In view of the importance of banana cultivation in India and fast growing tissue culture industry, it is necessary to have accurate, sensitive and reliable indexing procedures available for routine indexing. In present study a comparison of available techniques was done to standardize the most suitable, reliable and reproducible test procedure for indexing of banana streak viruses infection in field as well as tissue culture raised plants. Further for the first time we report the occurrence and distribution of banana streak disease in different regions of India through surveys done during 2010–2013.

2. Materials and methods

2.1. Survey, sample collection and electron microscopy

During 2010–2013, a survey was conducted in different banana fields of North East (Kahikuchi, Assam), North (Hamirpur and Bilaspur, Himachal Pradesh), East (Muzaffarpur, Bihar), West (Pune, Maharashtra) and South (Bagalkot and Belgaum, Karnataka; Virudhunagar, Tamil Nadu; Thrissur and Malappuram, Kerala) (Fig. 1a). Symptomatic as well as asymptomatic leaf samples were collected. Leaves were either used fresh or stored at −80 °C for further analysis. Different tissue culture raised batches of banana cultivar Grand Naine (AAA) in bottles as well as primary/secondary hardened plants received at Advanced Centre for Plant Virology, New Delhi during the years 2010–2013 were also used for the analysis. Leaf dip electron microscopy of fresh leaf samples was done to observe the associated virus particles. The grid was placed on a drop of leaf extract. After 1 min the grid was washed with 10 drops of distilled water and negatively stained with 2% aqueous uranyl acetate. Immediately after drying the grid was examined under electron microscope (JEOL 100 CX-11) at Advanced Centre for Plant Virology (ACPV), IARI, New Delhi.

2.2. Antigen coated plate-enzyme linked immunosorbent assay (ACP-ELISA)

ACP-ELISA was carried out for detection of BSV infection in the samples. The plates were coated with leaf extracts diluted 1:4 (w/v) in coating buffer (15 mM sodium carbonate, 35 mM sodium bicarbonate, 2% polyvinylpyrrolidone 40, pH 9.6) and incubated at 37 °C for 1 h. After three washings with PBST buffer (136 mM NaCl, 1.4 mM KH_2PO_4, 2.6 mM KCl, 8 mM Na_2HPO_4, 0.05% Tween-20, pH 7.4), the plates were blocked with 2% bovine serum albumin (BSA) for 2 h at 37 °C. After three washing with PBST, specific antiserum (in house developed at ACPV, IARI, New Delhi or commercial antiserum in some cases) diluted (1:1000) with PBSTPO (PBST with 2% polyvinylpyrrolidone 40 and 0.2% ovalbumin) was loaded to the plates and incubated at 37 °C for 1 h followed by three washing with PBST. Goat anti-rabbit IgG-AP conjugate (Sigma–Aldrich, St. Louis, MO: at dilution of 1:30,000 in PBSTPO) was added and incubated at 37 °C for 1 h. Finally, the plates were washed thrice with PBST and p-nitrophenyl phosphate (PNPP) substrate (0.5 mg/ml PNPP dissolved in 9.7% diethanolamine buffer, pH 9.6) was added. The OD_{405} values were measured by EPSON ELISA reader (TECAN A-5082, Sun Rise, Austria) after 30 min and 1 h of substrate addition.

2.3. DNA extraction and direct polymerase chain reaction (PCR)

Total plant DNA was extracted from 100 mg of fresh or frozen leaf samples either by CTAB method (Haible et al., 2006) or with the DNeasy Plant Mini Kit (Qiagen, Gmbh, Hilden, Germany) following the manufacturer's protocol. Direct PCR amplification was carried out using degenerate primers BadnaFP and BadnaRP, targeting conserved RT/RNase H region of badnaviruses genome following the protocol described earlier (Yang et al., 2003).

2.4. Duplex-immunocapture-polymerase chain reaction (D-IC-PCR)

For specific detection of BSMYV and BSOLV the two viruses reported from India (Selvarajan et al., 2008; Baranwal et al., 2014), specific primer pairs reported earlier (Geering et al., 2000) were used in D-IC-PCR following the protocol described earlier (Le Provost et al., 2006) with little modification. Sterile polypropylene microfuge tubes (Axygen, Union City, USA) were coated overnight at 4 °C with 25 μl of commercial BSV antiserum (Agdia) or polyclonal antiserum (in-house developed or the antiserum kindly provided by Dr. A.D.W. Geering) at a dilution of 1:250 or 1:500 in carbonate coating buffer (15 mM sodium carbonate, 34 mM sodium bicarbonate, pH 9.6), then washed with PBST buffer. For preparation of plant extracts 0.5 g leaf samples were ground in grinding buffer (2% polyvinylpyrrolidone 40, 0.2% sodium sulphite and 0.2% bovine serum albumin in PBST) at three different dilutions *viz.* 1:4, 1:8 and 1:10 (w/v). 25 μl supernatant of plant extracts was loaded in IgG-coated tubes and incubated at 37 °C for different durations ranging from 1 h, 2 h, 3 h, 5 h and overnight for immunocapture of virions. After washing the tubes thrice with PBST, followed by a washing with sterile distilled water, the multiplex PCR was carried out directly in tubes using degenerate primers or BSMYV/BSOLV specific primers (Geering et al., 2000) and *Musa* sequence tagged microsatellite primers AGMI025 and AGMI026 (Lagoda et al., 1998) as internal control, which are also able differentiate among A and