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Virus indexing in Banana: A review

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Abstract
Virus infections are a major biotic limitation for banana (*Musa* spp.) production because they reduce outp ut and restrict international germplasm movement. The banana bunchy top virus and banana streak viruse s are the most common and economically devastating viruses known to infect bananas. The most cost-effective way to reduce the harmful effects of viral infections on banana production is to employ virus-resistant bananas. The banana and plantain (*Musa* spp.) are India's most important fruit crops, providing a living for millions of resource-poor small farmers. For increased output, it is critical to use high-quality planting material. Although conventional suckers are still the most common planting material, tissue-culture plants are becoming more popular because to their benefits, such as more uniform bunches with even maturity and higher yield. For *Banana bunchy top virus* (BBTV) and *Cauliflower mosaic virus* (CMV), specific polyclonal antibodies were used in a double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA). Polymerase chain reaction (PCR)-based detection of *Banana Bract mosaic virus* (BBrMV) infected tissues confirmed the presence of the viruses in these plants. This article summarises current advances and future prospects in the detection of viruses in bananas.

Keywords: Banana, BBTV, BBrMV, PCR, ELISA

Introduction
Viral pathogens are major impediment within the banana tissue-culture (TC) industry. Among the viral diseases, BSV (*Banana streak virus*), BBr MV (*Banana bract mosaic virus*), CMV (*Cucumber mosaic virus*) and BBTV (*Banana bunchy top virus*) are occurring severely and causing major losses to banana farmers. Among the disparate native banana cultivars in Andhra Pradesh, Karpura Chakkarakeli (AAB) is one among the favoured banana cultivar and occupied 35,000 ha. Unfortunately, this cultivar is much succumbed to *Banana streak virus* (BSV), genus Badnavirus, the causal agent of viral leaf streak, is taken into account to be the foremost threatening and causing great yield loss. Within the light of severe spreading of the viral diseases during recent times there's an utmost have to increase the production and distribution of disease free planting material as per the demand which is raising day by day from the potential banana growing districts within the state. The realm expansion has however, been in the course of rampant spread of pests and diseases, most significant among which are viral diseases transmitted through non-indexed planting material. Additionally, symptoms of viral diseases seldom get confused with the nutrient deficiency symptoms. CMV and BSV infection are often confused due to induction of comparable symptoms. Thus, the requirement for production of disease free, quality planting material is being felt over ever before not only within the state but in other regions as well. Early detection by means of sensitive diagnostic techniques is the main way to stop them.

Development of tissue culture (TC) and *in vitro* plant propagation techniques have made possible to mass propagate top quality banana and plantain planting material. These advancements, termed as micro propagation, have led to the arrival of economic tissue culture industries dedicated to banana planting material production for domestic as well as international trade and have replaced conventional vegetative sucker production in many banana growing regions around the world (Israeli *et al*., 1995; Smith *et al*., 2005) [51, 32, 33]. While microproprogation offers several advantages over conventional sucker production but it doesn't exclude, the viruses, viroids, phytoplasma and fastidious bacteria, if present in the mother stocks (Diekmann and Putter, 1996) [50]. Propagation material derived from the infected mother stocks leads to perpetuation of pathogens resulting in low yields and poor quality fruits, additionally; infected material is serves as vehicles for spread of pathogens which is a major concern for domestic and international movement of the planting material.

Infected planting material established within the fields isn't amenable to curative procedures and that they act as sources for secondary spread of pathogens by natural vectors like aphids, beetles, mealybugs and also through agriculture implements. This risk of pathogen spread through planting material is of a high concern because banana is pretentious by several important pathogens of high quarantine significance. Different diagnostic techniques *viz*, Polymerase chain reaction (PCR), reverse transcriptase PCR (RT-PCR), non-radioactive probe-based nucleic acid spot hybridisation (NASH) and enzyme-linked immune-sorbent assay (ELISA) based techniques were developed and utilized for indexing.

Indexing techniques for banana viruses

Serological Detection Techniques Various types of serological assays are currently available for all seven known banana viruses. Enzyme Linked Immuno Sorbent Assay (ELISA) tests with monoclonal antibodies (Mabs) are commonly used for the accurate detection of BBTV (Wu and Su, 1990; Thomas and Dietzgen, 1991; Geering and Thomas, 1996; Espino *et al*., 1989) [27, 14, 11]. Geering and Thomas (1996) [14] amplified triple antibody sandwich (TAS)-ELISA method for routine virus indexing of *Banana Bunchy Top Virus* (BBTV). The plate-trapped antigen (PTA)-ELISA method detected BBTV by monoclonal antibodies (Wu and Su, 1990) [27] produced and. Wanitchakorn *et al*. (1997) [48] amplifiedthe recombinants expressing BBTV coat protein and produced the polyclonal antiserum which effectively detected the virus in asymptomatic plants. Selvarajan *et al*. (2002) [43] produced polyclonal antiserum for BBTV isolate and reported that the direct antigen coating (DAC)-ELISA method was more sensitive than dot immuno binding assay (DIBA) for detection of BBTV. BBrMV has been detected by serology using ELISA (Espino *et al*., 1990; Singh *et al*., 2000; Thomas *et al*., 1997; Selvarajan *et al*., 2006b) [12, 24, 25, 23]. Espino*et al*. (1989) [11] amplified monoclonal antibodies for BBrMV and detected the virus by double antibody sandwich (DAS)-ELISA method. The coat protein of BBrMV was expressed in Escherichia coli as a fusion recombinant protein utilized to produce a high-titre BBrMV-specific polyclonal antiserum for serological assays (Rodoni *et al*., 1997) [41]. Detection of BSV has been problematic because of serological and genomic heterogeneity of virus isolates (Lockhart and Olszewski, 1993) [38]. Thottappilly *et al*. (1998) [47] bringabout the production of high-titre polyclonal antibodies against Nigerian isolates of BSV. They reported that TAS-ELISA was more sensitive than antigen-coated plate (ACP) - ELISA and protein-A coated antibody sandwich trapped (PAS)-ELISA. Agindotan *et al*. (2003) [28] reported high-titred monoclonal antibodies for BSV, which may detect all the isolates of BSV. Agindotan *et al*. (2006) [29] reported that IC-PCR was considerably more sensitive than immune electron microscopy (IEM) for detecting typical BSV, while IEM proved to be of compareble sensitivity as TAS-ELISA by sap dilution end-point analyses. Kiranmai *et al*. (1996) [17] have demonstrated potential applicability of DAC-ELISA in large scale indexing of banana for CMV infection.

The detection of BBTV in tissue-culture samples which is equally sensitive as Polymerase Chain reaction (PCR)Nucleic Acid Spot Hybridisation (NASH) technique is used. The similar technique has been applied for detection of BBrMV, CMV and BSMysV (R. Selvarajan, unpublished). The 32P and digoxigenin (DIG)-labelled probes were utilized for the detection of BBTV in Australia Xie and Su (1995). Kiranmai *et al*. (1998) [36, 37] used dot blot hybridisation technique to detect CMV banana isolate with 32P-labelled radioactive probe also with DIG-labelled probes. Heterologous 32P-labelled probe prepared for CMV infecting pepper successfully detected the CMV-banana isolate (Srivastava *et al*., 1995) [45]. DIG-labelled non-radioactive DNA probe has been accustomed to detect CMV in sap extracted with pinpricking the pseudostem. This method is less complicated and fewer expensive than routine time-consuming preparation of extracts (Kiranmai *et al*., 1998) [36, 37].

Polymerase Chain Reaction (PCR) PCR-based detection systems are now also available for all banana viruses (Dietzgen *et al*., 1999; Harper *et al*., 1999) [32, 33, 34]. Xie and Hu (1995) [49] used PCR for detecting the Hawaiian isolates of BBTV, and it had been 1000 times more sensitive than ELISA or dot blots with DNA probe. A simple, single-step plant-tissue preparation protocol to scale back plant inhibitory factors interfering with PCR suitable for the detection of BBTV in corm, leaf and root tissues by PCR was developed (Thomson and Dietzgen, 1995) [46]. Mansoor *et al*. (2005) [40] detected a Pakistan isolate of BBTV by PCR and used primers for banana genomic sequences as an inner control for overcoming the uncertainty over inherent PCR. Selvarajan *et al*. (2007) [42] also developed a PCR-based detection method for Indian isolates of BBTV. PCR has been exploit to detect BBTV from viruliferous aphids (Manickam *et al*., 2002; Selvarajan *et al*., 2006b) [39, 23]. BBrMV was detected by RT-PCR in total nucleic acid extracts from infected plants, using specific or degenerate potyvirus group primers (Bateson and Dale, 1995; Thomas *et al*., 1997) [30, 25]. Indian isolates of BBrMV were detected from pseudostem and banana bracts through reverse transcription (RT) - PCR (Sankaralinkam*et al*., 2006; Selvarajan *et al*., 2006b) [23]. A Kerala isolate of BSV was detected by PCR using primers specific to conserved domains of RT/RNaseH region of the genome of badnavirus (Cherian *et al*., 2004) [31]. Singh *et al*. (1995) [44] and Hu *et al*. (1995) used RT-PCR reaction assay for detection of CMV infecting banana. Detection of banana streak virus (BSV) and its serological relationship with other banana viruses by ELISA (Manoranjitham *et al*., 2019) [52]. Molecular cloning and characterization of coat protein gene of banana bract mosaic virus affecting banana cv. Mysore Poovan (ABB) showed BBrMV infected samples by DAC-ELISA (Darshan *et al*., 2019) [7, 8].

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