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Molecular Identification of Banana Streak Virus in Banana through PCR

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A B S T R A C T

Banana is the most important fruit crop such as rice, wheat, and maize. Banana (*Musa* spp.) is cultivated in more than 130 countries in the tropics and subtropics and is a staple food crop for millions of people. Its cultivation is affected by various diseases. Among them BSD (Banana Streak disease) caused by (Banana streak Virus) is one of the most important constraints in banana production worldwide. BSV is the causal agent of this viral disease in Banana crop (Musa spp.) It is a member of the family Caulimoviridae, genus Badnavirus. Badnaviruses have bacilliform-shaped virions, =30 X 150 nm in size and a circular, noncovalently closed dsDNA genome. BSV has a monopartite genome of ~ 7.2-7.8 Kb encoding three open-reading frames (ORF_s). And causes heavy economic loss in banana cultivated areas after showing chlorotic and necrotic streaks on leaves. On the basis of symptoms such as green streaks on pseudostem, stunted growth, distorted fruit in smaller branches, leaf samples were taken for isolating genomic DNA using CTAB method with some modification in Selvarajan protocol. DNA was quantified (50ug/lit DNA). PCR amplification was performed on all isolates from IARI, New Delhi, Hastinapur (UP), and Healthy sample for early detection of virus. PCR done with the help of specific primer forward (BSMysV & RNase H 5466) and reverse (BSMysV & RNase H 6196) primers, using total extracted DNA. The Agarose gel was loaded with ladder DNA (1kb), followed by sample DNA. Among DNA samples, all showed amplification by specific primer pair of BSV. During the study it has been clear that the isolates of Meerut and IARI New Delhi were infected by BSV. So, based on this analysis, it can be concluded that, detection of virus early and proper eradication of infectious plants is very important before passing of virus to another healthy plants.

Introduction

Banana is one of the most important fruit crops such as rice, wheat, and maize. Banana (*Musa* spp.) is cultivated in more than 130 countries in the tropics and subtropics and is a staple food crop for millions of people, particularly in Africa (Frison and Sharrock, 1998). Banana is one of the important fruit crops grown in India; India is the largest producer of banana and plantains. Although India contributing nearly one-third of the global banana production. The area of India under banana cultivation is about (830.00 million hectare), which shares about 29.19% in world total production.

Infact during fiscal year 2020, volume of banana produced across India accounted for around 32 million metric tons. This was an increase of about one million metric tons from the previous fiscal year. The major banana growing states are; Andhra Pradesh, Assam, Bihar, Gujarat, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Odisha, Tamil Nadu, West Bengal, and Uttar Pradesh. Among the banana growing states, Tamil Nadu is the largest producer in India with maximum production of 8.25 million tons from area of 0.125 million ha. banana contain fiber, potassium, vitamin C, and B_6. Banana genotypes potential is greatly hampered by several biotic and abiotic constraints prevailing in different banana producing regions. Among them, Fungal, bacterial and viral diseases have been threatening banana production in Uttar Pradesh (India).Viruses are considered a serious threat and play a major role in yield loss. Banana and other *Musa spp*. are also affected by five major viruses. These are *Banana bunchy top virus* (BBTV)genus *Babuvirus*; *Banana streak virus* (BSV) genus *Badnavirus*, *Cucumber mosaic virus* (CMV) genus *Cucumovirus*, *Banana bract mosaic virus* (BBrMV) genus *Potyvirus* and *Abaca mosaic virus* (AbaMV) genus *Potyvirus.* Recently, there are other viruses that were identified namely *Banana mild mosaic virus* (BanMMV) and *Banana die-back virus* (Pietersen and Thomas, 2000).

Banana streak disease was first reported from Ivory coast in 1968.and BSV is the causal agent of this viral disease in Banana crop (*Musa* spp.). The most characteristic disease symptoms on the leaves of banana and plantains are chlorotic and necrotic streaks, which tend to be intermittent in expression. Diseased plants may be stunted, and fruit may be distorted with a thinner peel and occur in smaller bunches. Banana Streak virus (BSV), the causal agent of the disease, was first isolated and characterized from banana in Morocco. BSV has affected banana production in countries like India, South Aisa, America etc. BSV is a member of the family Caulimoviridae, genus Badnavirus. Badnaviruses have bacilliform-shaped virions =30 X 150 nm in size and a circular, noncovalently closed dsDNA genome. BSV has a monopartite genome of ~ 7.2-7.8 Kb encoding three open-reading frames (ORF_s). The disease, which is known to affect only Musa is spread by vegetative production, mealybugs and through seed. BSV and nine other species of the other 11 definitive members of the genus Badnavirus are transmitted by mealybugs (Pseudococcidae) (Lockhart and Olszewski, 1994). Mealybug vectors of badnavirus include species of Pseudococcus, Planococus, Planococcoides, Ferrisia, Saccharicocus and Dysmicoccus (Brunt, 1970; Lockhart *et al.*, 1992). BSV has been shown to be transmitted by Planococus citri and *Saccharicoccus sacchari*, both of which colonize banana (Lockhart *et al.*, 1992).There are different techniques used to measure DNA naming ISEM (Immunosorbent electron microscopy, ELISA (Enzyme-linked immunosorbent assa), using polyclonal antisera raised against a mixture of BSV antigens.PCR (Polymerase chain reaction) done by using forward (BSMysV& RNase H 5466) and reverse (BSMysV& RNase H 6196) primers. From above we particularly used the PCR technique for the identification of Banana Streak Virus (BSV).Three BSV species are currently recognized: Banana streak Mysore virus (BSGFV), Banana streak OL virus (BSOLV), and Banana streak acuminate Vietnam virus (BSAcVNV). For the management of viral disease, early detection is the only way as once the plant is infected with virus, there is no cure. Therefore, there is a need of development of single tube multiplex PCR based techniques for the simultaneous detection of multiple virus infecting bananas with the aim to Survey and sample collection of bsv infected plant from Delhi, UP.

Materials and Methods

The experiment on “Polymerase Chain Reaction based detection of Banana streak virus (BSV)” was carried out at the Department of Genetics and Plant Breeding, Chaudhary Charan Singh University, Meerut (U.P.) during 2020-21. Details regarding the experimental materials used and methodology adopted for various experiments are presented below.

Parent Material

Leaf samples were collected from plants in and around Meerut district where Banana streak disease (BSV) were prevalent. Leaf samples were collected from those plants which showed the symptoms as described by Iskra Caruna (1900) where the symptoms consist of broken or continuous streaks which vary in colour from yellow to brown to black, necrosis of emerging leaves, internal necrosis of the pseudostem and plant death (Lockhart, 1995). Samples from apparently healthy plants were also collected. The collected samples were stored in the freezer at -20^{0}C and used for future molecular and serological assays.

Isolation of genomic DNA

Total genomic DNA from healthy and infected banana leaves was isolated using the Protocol Selvarajan *et al.,* (2002) with some modification in it. The preserved leaves (2 grams) were taken and washed in distilled water and taken in mortar pestle. To the leaf samples 1 ml of CetylTrimethyl Ammonium Bromide (CTAB) extraction buffer, 6.5 ml of solution II and 500 micro litre of 20 per cent SDS was added and leaf sample was crushed into a fine powder using frozen liquid nitrogen. After vigorous shaking the mixture was subjected to incubation at 65^{0}C for 1 hour. Then to the mixture 5 ml of chloroform: isoamyl alcohol (24:1) was added and mixed gently by inversion for 15 minutes and centrifuged at 10,000 rpm, 4^{0}C for 15 minutes. To the aqueous phase 400 micro litre of ice cold isopropanol was added and mixed gently and subjected to centrifugation at 10,000 rpm, 4^{0}C for 15 minutes. Precipitated DNA was washed with 70 per cent ethanol and air dried. To the pellet obtained 4 micro litre RNase A was added and incubated at 37^{0}C for 1 hour. Then equal volume of phenol: Chloroform: isoamyl alcohol (25:24:1) was added and centrifuged at 13,000 rpm, 10^{0}C for 5 minutes. The aqueous phase was transferred into a fresh 2 ml eppendorf tube and equal volume of chloroform: isoamyl alcohol (24:1) was added and centrifuged at 13,000 rpm, 10^{0}C for 5 minutes. The supernatant was collected and to it one tenth volume 3M sodium acetate (pH 5.2) was added and stored at -20^{0}C for 1 hour. The precipitate was centrifuged at 10000 rpm, 4^{0}C and washed twice with 70% ethanol and dissolved in 500 micro litre TE buffer (pH 8.0) and stored at -20^{0}C.

Quantification of DNA

DNA quantification was carried out with the help of UV-spectrophotometer. The optical density (O.D.) of the DNA sample dissolved in TE buffer was recorded at both 260 nm and 280 nm. Since the O.D. of 1.0 at 260 nm represent 50 microgram/litre of DNA, the quantity of DNA in the sample was estimated by employing the following formula:

Concentrated of DNA (microgram/litre) = A260 Multiply 50 multiply Dilution factor (where, A260 is absorbance at 260 nm). The quality of DNA at ratio of 1:8 indicates good quality DNA.

Detection of BSV by PCR

The sequence of the protein BSMysV-F and RNAse H-BSV5446 gene was taken from Gene bank database of NCBI (National Centre for Biotechnology Information). The primer was designed manually using DNA sequence 18 nt- 30nt with good GC content greater than 50.0 per cent. The primer is specific for the coat protein gene of BSV obtained from the ORF III of the virus. The sequences of these primers and the size of amplicon are listed in the (table 1).all these primers were designed to amplify the fragment of 1kb.PCR reaction was done using 20 ul of reaction mixture containing 2 ul of template DNA, 2.5 ul 10x buffer, 2 ul dNTP mix, 1ul $Mgcl_2$, 2 ul forward (BSMysV & RNase H 5466), 2 ul reverse (BSMysV & RNase H 6196) primers, 1 ul Taq DNA polymerase, 7.5 sterile water. this mixture was set in a Programmed thermal cycler (Thermo fisher scientific Veriti) and PCR was carried out by initial Denaturation of the template DNA at 94^{0}C for 4 min followed by 30 cycles of Denaturation at 94^{0}C for 30 sec, annealing at 52^{0}C for 2 min and extension at 72^{0}C for 2 min

with final extension at 72^0C for 10 min. the amplified products were analysed by electrophoresis using 1.5% agarose in 1xTAE buffer with Ethidium bromide(5ul) and electrophoresed at 70-80 volt for 30-40 min (Electrophoresis unit Genetix).the gels were visualized on a UV-transilluminator and gel documentation system (Nugenus syngene system).1 kb DNA Ladder was used as size marker. The PCR amplified products were purified using MinElute gel extraction kit (Thermo scientific).and the purified DNA was stored at -20 °C to be send for Sequencing.

The PCR conditions of both primers are given below separately and their amplification stages are mentioned.

Results and Discussion

Symptoms of necrotic streaks, stunted growth, distorted fruit and smaller bunches were observed during a survey in field (Hastinapur, U.P.), and IARI, New Delhi (2019-2021). Chlorotic and necrotic streaks were seen after infection.

The presence of BSV in all samples from the symptomatic plants was confirmed by PCR amplification of about kb products with BSV CP and PR primer. No amplification was observed in any of the non-symptomatic Banana samples.

Bananas (Musa spp.) are grown as staple food, important cash crop and major export crop in many of the tropical and subtropical areas of the world including India (Annual report INIBAP, 1992). The plants of banana grown at commercial level are basically infertile and are propagated vegetatively, traditionally using suckers. However, recently, for large scale planting of banana, many commercial operators have adopted tissue culture method. Banana is normally affected by four viruses namely, BSV, BBTV, BBrMV CMV (Burns *et al.,* 1994; Diekmann and Putter, 1996 and Thomas *et al.*, 1997). These viruses are radially transmitted through vegetative propagules (Diekmann and Putter, 1996) and therefore cause threat to the production in areas where viruses are endemic as well as in areas that are virus free. No effective resistance is known in Musa to any of these viruses. So infection can be control by the use of virus plants. Identification of BSV infection in the suckers is very difficult as the symptom development is not visible at early stage of infection. Immunological detection using ELISA technique is not effective as the virus is present in low tire in the banana plant. There is only one successful report of ELISA-based detection of BSV. Many of the recent literature free planting material, implementation of quarantine barriers and rouging of infected showed that BSV detection was effective in using PCR method PCR method had the advantage of amplifying the target nucleic acid present even at very low level and it had become an attractive technique for the diagnosis of much plant viral disease. The CTAB method was used to isolate good quality of DNA was extracted using Sarcosil chemical and modified CTAB method. PCR screening revealed BSV infection in four plants and this result was supported by the development of BSV symptoms. Therefore, PCR based method using CP and PR primers was better for the early detection of the disease in banana plants and could be effectively utilized for screening of streak virus. Consequently, all above results confirmed that the PCR condition including the DNA from the banana sample was good enough for the PCR amplification. Gel Electrophoresis and DNA screening of samples from infected plants using PCR showed diagnostic method could be used to make diagnostic tool or kit for the identification of virus in banana and further research.

Table.1 Primers used for amplification of DNA sequence of BSV by PCR

Primer pair		Nucleotide sequence	Expectedsize
BSMysV-F	Forward	5'TAAAAGCACAGCTCAGAACAAACC3'	~589 bp
BSMysV-R	Reverse	5'CTCCGTGATTTCTTCGTGGTC-3'	
RNAseH-BSV5446 –F	Forward	5'AGAGTGGGTTTCATCAAGTAGC-3'	~750
RNAseH-BSV5446-R	Reverse	5'GAATTTCCCGCTCGCATAAG-3'	

Fig.1 PCR Conditions of BSMysV

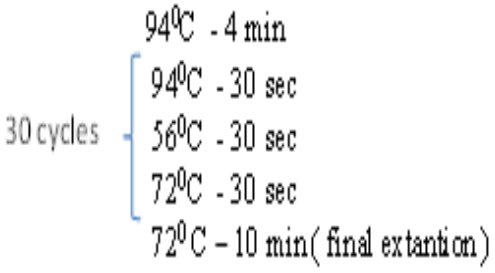


Fig.2 PCR Condition of RNaseH

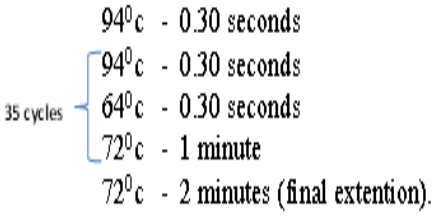
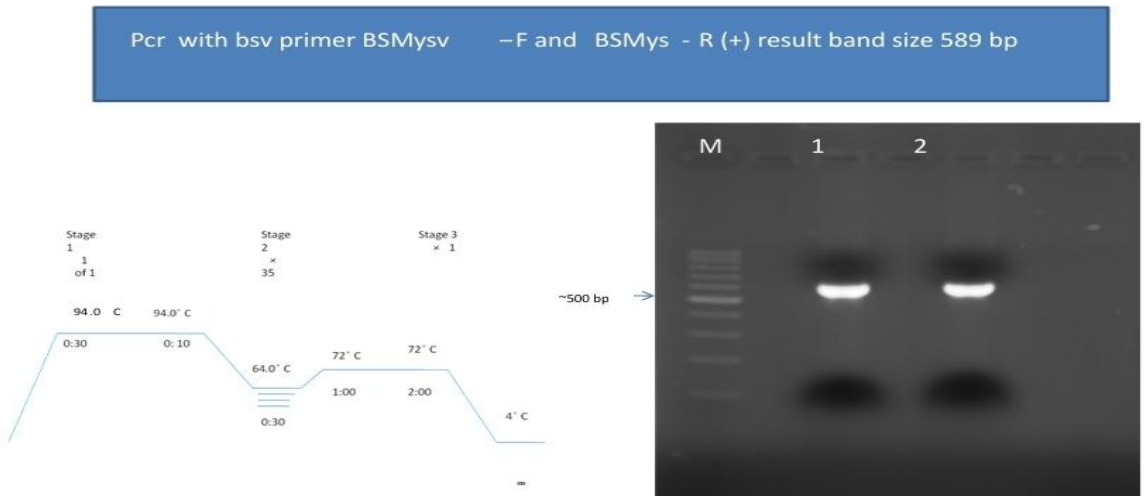


Fig.3 Sample from IARI, New Delhi

Fig.4 Sample from Mawana Meerut

Fig.5 PCR amplification of infected samples by primer pair BSMysv M: 100 bp DNA marker; 1: BSV infected IARI sample 1, 2: BSV infected IARI sample 2.



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