International Journal of Agriculture, Environment & Biotechnology
Citation: IJAEB: 7(4): 765-767 December 2014
DOI Number: 10.5958/2230-732X.2014.01385.0

GENETICS AND PLANT BREEDING

Polymerase chain reaction based detection of banana bunchy top virus using coat protein based primers

R. K. Ponsubrya, Swapna Alex*, K. Rajmohan and K. B. Soni

Department of Plant Biotechnology, College of Agriculture, Vellayani, Thiruvananthapuram, Kerala-695 522, India.

*Corresponding author: alexswapna@hotmail.com

Paper No. 266 Received: 12 February, 2014 Accepted: 16 November, 2014 Published: 23 December, 2014

Abstract

Banana Bunchy Top virus (BBTV) is a virus seriously affecting banana cultivation. Primers were designed for the amplification of coat protein gene for the diagnosis of BBTV in the commonly grown banana cultivars of Kerala, India, viz., Red Banana (AAA), Palayankodan (AAB), Dwarf Cavendish (AAA), Motta Poovan (AAB) and Ney Poovan (AB). PCR detection using these primers at an early stage can aid in disease free planting material production.

Highlights

- Primers designed for the early detection of Banana bunchy top viral infection using PCR produced an amplicon of size 500 bp in all the infected samples of the five varieties of banana.

Keywords: PCR primers, BBTV detection, banana

Banana Bunchy Top Virus (BBTV) is one among the four major viruses seriously affecting banana plantations in India (Selvarajan *et al*., 2010; Banerjee *et al*., 2014). The virus belongs to the family Nanoviridae and contains a multi-component genome of circular ssDNA (Vishnoi *et al*., 2009). The virus is transmitted by an aphid vector, *Pentalonia nigronervosa* Coq. (Hu *et al*., 1996, Hooks *et al*., 2009; Chen and Hu, 2013). The infection is a great hindrance in the multiplication and supply of quality planting material both by conventional and *in vitro* propagation approaches. Since the primary source of infection of BBTV is through infected suckers, it is necessary to detect the presence of the virus in suckers before they are planted or used for micropropagation. Diagnosis of this viral disease is usually carried out by Enzyme-linked immunosorbent assay (ELISA). However this technique is not sufficient to detect the virus in the very early stages. Techniques like Polymerase Chain Reaction (PCR) can detect the virus even at very minute concentrations (Galal, 2007; Joshi and Deshpande, 2011).

In the present study, primers were designed for the amplification of coat protein gene for the diagnosis of BBTV in the commonly grown banana cultivars of Kerala, viz., Red Banana (AAA), Palayankodan (AAB), Dwarf Cavendish (AAA), Motta Poovan (AAB) and Ney Poovan (AB).

Materials and Methods

Primer Designing and Synthesis

The sequences of the coat protein gene with the accession numbers EF584544, AY272038, AY953428, DQ515970 and AY534140 coding for BBTV coat protein gene were retrieved from the NCBI Genbank. Conserved regions were obtained using multiple sequence alignment tool Clustal X (1.8). The primers

were designed using the Primer 3 algorithm and were analyzed by BLAST N (Altschul *et al*., 1997) to find the homology of the primers. Oligonucleotide properties were calculated using the program Oligocalc (www. Basic.northwestern.edu/biotools/oligocalc.html) for its performance. The DNA oligos were synthesized by Sigma Aldrich (USA).

DNA Extraction And PCR Amplification

Leaf samples were collected from the healthy and symptomatically BBTV infected plants (infection confirmed by ELISA) from the cultivars viz., Red banana (AAA), Palayankodan (AAB), Dwarf Cavendish (AAA), Motta Poovan (AAB) and Ney Poovan (AB).

Total genomic DNA was isolated using the protocol developed by Doyle and Doyle (1990). The quality of the DNA isolated was checked by taking the readings at 260 and 280 nm in UV-Vis spectrophotometer (BioRad, USA).

The polymerase chain reaction was performed with 20 ng of genomic DNA as template, 0.75U of Taq DNA polymerase (Bangalore Genie), 1X PCR buffer, 100 μM of dNTPs and 200 pM each of forward and reverse primer in a final volume of 25 μl in thermal cycler (BioRad). The PCR cycles started with an initial denaturation step at 94°C for 2 min, followed by 30 cycles of denaturation at 94 °C for 45 sec, annealing at 40 °C for 30 sec and extension at 72 °C for 1 min. The reaction was terminated by a final extension step at 72 °C for 5 min.

PCR amplified products were subjected to electrophoresis in a 2 % agarose gel in 1X TBE buffer at 100 volts for 2.5 h. A 1kb DNA marker was also loaded. The ethidium bromide stained gels were documented using Gel Documentation system (BioRad, USA).

Results and Discussion

PCR amplification depends on quality of the isolated DNA (Das *et al*., 2009). In the present study the protocol of Doyle and Doyle (1990) yielded good quality DNA with A260/280 ranging from 1.7 to 1.9 and hence can be effectively used for PCR amplification.

In this study, the sequences of the forward and reverse primers designed based on the coat protein gene sequences of Banana bunchy top virus using the Primer 3 Algorithm are 5′ATGGCTAGGTATCCGAAGAAATCC and 5′ A C T C C A G A A C T A C A A T A G A A T G C C respectively. The oligonucleotide calculation program used for the analyses of the primers showed no hairpin loop formation, no 3′ complementarity and no self annealing, indicating good quality (Chuang *et al*., 2013). On PCR amplification using the designed primers an amplicon of the size approx. 500 bp was obtained in all the infected samples (Plate). Reproducibility of the reaction was also confirmed.

Transmisssion through infected planting material is a major cause of spread of banana bunchy top virus (Harding *et al*., 2000; Galal *et al*., 2007). PCR is an efficient method for BBTV detection and hence can be used as a reliable detection procedure for the production of virus free planting material (Chandrashekar *et al*., 2011). Primer designing is most important for the successful detection of the virus using PCR (Abd-Elsalam, 2003). Differences in the coat protein gene sequences have been reported in the virus isolates from different regions (Selvarajan *et al*., 2010). So it is important to develop primers specific to each region specific isolate to make the detection procedure more accurate. Earlier reports indicate PCR Detection of Banana Bunchy Top Virus (BBTV) at tissue culture level in *Musa spp*. cultivars 'Virupakshi' and 'Sirumalai' (AAB) from Tamil Nadu (Mahadev *et al*., 2013). However, there are no reports on PCR primers designed for coat protein from banana cultivars of Kerala. In this context, the primers designed against the coat protein gene in the present study can be used for the early detection of BBTV using PCR in banana cultivars of Kerala enabling the production of virus free plantlets by micropropagation. The amplified fragments can be sequenced and used for designing of multiplex primers for easier detection of viral infection.

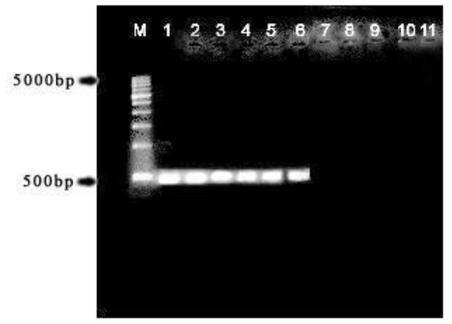

Plate Amplification using BBTV coat protein gene primer

Lane M 500 bp marker
Lane 1 Red Banana -infected
Lane 2 Palayan Kodan-infected
Lane 3 Dwarf Cavendish infected
Lane 4 MottaPoovan infected
Lane 5 Motta Poovan infected
Lane 6 Ney Poovan infected
Lanes 7-11 Healthy samples

Conclusion

The primers designed in the present study can be used in PCR for the early detection of banana bunchy top virus infection in the *Musa spp*. cultivars of Kerala *viz*., suckers of Red Banana (AAA), Palayankodan (AAB), Dwarf Cavendish (AAA), Motta Poovan (AAB) and Ney Poovan (AB).

References

Abd-Elsalam, K.A. 2003. Bioinformatic tools and guideline for PCR primer design. *African Journal of Biotechnology* **2**: 91-95.

Altschul, S.F., Madden, T.L. Schäffer, A.A. Zhang, J. Zhang, Z. Miller, W. and Lipman, D.J. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research* **25**: 3389-3402.

Banerjee, A., Roy, S., Behere, G.T. Roy, S.S. Dutta, S.K. and Ngachan, S.V. 2014. Identification and characterization of a distinct banana bunchy top virus isolate of Pacific-Indian Oceans group from North-East India. *Virus Research* **183**: 41-49.

Chandrasekar A., K. Kalaiponmani, S. Elayabalan, K.K. Kumar, K. Angappan and P. Balasubramanian. 2011. Screening of banana bunchy top virus through multiplex PCR approach, *Archives of Phytopathology and Plant Protection* **44**: 1920-1925.

Chen Y., and X. Hu. 2013. High-throughput detection of banana bunchy top virus in banana plants and aphids using real-time TaqMan® PCR. *Journal of Virological Methods* **193**: 177–183.

Chuang L.Y., Cheng, Y.H. and Yang. C.H. 2013. Specific primer design for the polymerase chain reaction. *Biotechnology Letters* **35**:1541-1549.

Das B.K., Jena R. C. and K.C. Samal. 2009. Optimization of DNA isolation and PCR protocol for RAPD analysis of banana / plantain (Musa spp.) *International Journal of Agriculture Sciences* **1**: 21-25.

Doyle, J. J., and J. Doyle. 1990. Isolation of plant DNA from fresh tissue. *Focus* **12**: 13-15.

Galal, A.M. 2007. Use of Polymerase chain reaction for detecting Banana bunchy top nanovirus. *Biotechnology* **6**: 53-56.

Hooks C.R., S. Fukuda, E.A. Perez, R. Manandhar, K.H. Wang, M.G. Wright, and R.P. Almeida. 2009. Aphid transmission of Banana bunchy top virus to bananas after treatment with a bananacide. *Journal of Economic Entomology* **102**: 493-499.

Harding R.M., A.S. Sadik, A. Bahieldin and J.L. Dale. 2000. A sensitive detection of banana bunchy top nanovirus using molecular genetic approaches. *Arabian Journal of Biotechnology* **3**:103-114.

Hu, J.S., M. Wang, D. Sether, W. Xie, and K.W. Leonhardt. 1996. Use of polymerase chain reaction (PCR) to study transmission of banana bunchy top virus by the banana aphid (*Pentalonia nigronervosa*). *Annals of Applied Biology* **128**: 55-64.

Joshi, M., and Deshpande, J.D. 2011. Polymerase Chain Reaction: Methods, principles and acpplication. *International Journal of Biomedical Research* **7**: **81-89 .**

Mahadev, S.R., Thamilarasan, S.K. and Kathithachalam A. 2013. PCR detection of Banana Bunchy Top Virus (BBTV) at tissue culture level for the production of virus-free planting materials. *International Research Journal of Biological Sciences* **2**: 22-26.

Selvarajan, R., M .M. Sheeba, V. Balasubramanian,, R. Rajmohan, N. L. Dhevi, and T. Sasireka. 2010. Molecular characterization of geographically different Banana bunchy top virus isolates in India. *Indian Journal of Virology* **21**: 110-116.

Vishnoi R., Shri, K.R. and Vivek, P. 2009. Molecular characterization of an Indian isolate of Banana bunchy top virus based on six genomic DNA components. *Virus Genes* **38**: 333-334.