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Identification and characterization of a distinct banana bunchy top virus isolate of Pacific-Indian Oceans group from North-East India

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ABSTRACT

Banana bunch top virus (BBTV) is considered to be a serious threat to banana production. A new isolate of the virus (BBTV-Umiam) was identified and characterized from local banana mats growing in mid-hills of Meghalaya in North-East India. The complete nucleotide sequence analysis revealed the presence of six full-length ssDNA components (DNA R, DNA U3, DNA S, DNA M, DNA C and DNA N) sharing major common region (CR-M) and a stem-loop common region (CR-SL). BBTV-Umiam showed a unique deletion of 20 nucleotides in the intergenic region of DNA R, the absence of predicted open reading frame (ORF) in DNA U3 and probability for a small ORF in DNA U3 expecting functional evidence at transcriptional level. Phylogenetic analysis based on 88 complete nucleotide sequence of BBTV DNA R available in GenBank generated two broad clusters of Pacific-Indian Oceans (PIO) and South-East Asian (SEA) groups including BBTV-Umiam within PIO cluster. However, BBTV-Umiam was identified as the most distinct member of the PIO group with 100% bootstrap support. This was further supported by the phylogenetic grouping of each genomic component of BBTV-Umiam at the distant end of PIO group during clustering of 21 complete BBTV sequences. BBTV-Umiam shared relatively less nucleotide identity with PIO group for each genomic component (85.0–95.4%) and corresponding ORF (93.8–97.5%) than that of earlier PIO isolates (91.5–99.6% and 96.0–99.3%, respectively). Recombination analysis revealed two intra-component and five inter-component recombination events in BBTV-Umiam, but none of them was unique. Moreover, the isolate was identified as major parental sequence for intra-component recombination event spanning the replication-associated protein encoding region in Tongan BBTV DNA R. The current study indicated differential evolution of BBTV in North-East India (Meghalaya). The natural occurrence of hybrids of *Musa balbisiana* and *M. acuminata* in this geographically isolated region could be the contributing factor in accumulating genetic distinctiveness in BBTV-Umiam which need further characterization.

1. Introduction

Banana bunchy top virus (BBTV), the causal agent of banana bunchy top disease (BBTD), has been considered as a serious threat to banana (*Musa* spp.) production in the Pacific, South and South-East Asia and Africa (Dale, 1987; Kagy et al., 2001; Kumar et al., 2011; Thomas et al., 2000). BBTV is the type member of the genus *Babuvirus* in the family *Nanoviridae* (Fauquet et al., 2005). BBTV Infection causes plant stunting, leaf bunching and impaired fruit development. The virus is transmitted by banana aphid (*Pentalonia nigronervosa*) in a persistent manner (Magee, 1940). The disease is considered to be spread at long distances by the movement of infected planting materials. Once established, the disease is difficult to eradicate and manage.

BBTV has a multipartite genome consisting of six circular ssDNAs (each approximately 1.1 kb long; Burns et al., 1995; Harding et al., 1991, 1993; Karan et al., 1997) those are individually encapsidated within separate icosahedral virions (each about 18–20 nm in diameter; Wu and Su, 1990). Generally, each of the six ssDNA components have an open reading frame (ORF) encoding different protein in the virion-sense strand such as rolling-circle replication initiation protein (Rep; encoded on DNA R) (Beetham et al., 1997), a protein with unknown function (encoded on DNA U3) (Beetham et al., 1999; Tian and Zhuang Liu, 2005), a capsid protein (CP; encoded on DNA S) (Wanitchakorn et al., 1997, 2000a), a movement protein (MP; encoded on DNA M) (Wanitchakorn et al., 2000b), a cell cycle link protein (Clink; encoded on DNA C) (Wanitchakorn

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et al., 2000b), a nuclear shuttle protein (NSP; encoded on DNA N) (Wanitchakorn et al., 2000b). All the components contain a highly conserved, major common region (CR-M) and a stem-loop common region (CR-SL) downstream of CR-M (Burns et al., 1995). In addition to the six integral DNA components, few BBTV isolates also carry a satellite DNA component that encodes a protein homologous to Rep encoded in DNA R (Horser et al., 2001).

Earlier studies have categorized BBTV isolates into two different lineages (South Pacific group and Asian group) on the basis of phylogenetic relationships amongst DNA R component sequences having ~1.9–3.0% intra- and ~10% inter-group sequence diversity (Karan et al., 1994). Recently, this grouping have been modified as Pacific-Indian Oceans (PIO) group (comprising the isolates from Australia, Egypt, Hawaii, India, Myanmar, Pakistan, Sri Lanka and Tonga) and South-East Asian (SEA) group (comprising the isolates from China, Indonesia, Japan, Philippines, Taiwan and Vietnam), respectively to reflect the expanded geographical distribution of the BBTV isolates (Yu et al., 2012) (Supplementary Fig. S1). It was proposed that the timescales over which these evolutionary groups diverged might span the history of banana cultivation (Perrier et al., 2011). However, several recent studies suggested that the two main BBTV lineages could have split only hundreds of years ago as an outcome of high basal mutation rate and frequent homologous recombination between different components of the same genome (Hu et al., 2007) as well as between homologous components in different genomes (Hu et al., 2007; Hyder et al., 2011).

The BBTV isolates reported so far from India have been characterized as the members of the PIO group (Karan et al., 1994; Selvarajan et al., 2010; Vishnoi et al., 2009). Only two complete BBTV genome (comprising all six components) sequences have been reported from India, one from the northern (Lucknow) and another from southern (Tamil Nadu) parts of India. The north-eastern (NE) region of India is a bio-diversity hotspot and possesses diverse germplasm of banana (both wild and cultivated). However, little is known about the molecular characteristics of BBTV occurring in the NE India, except the preliminary information on BBTV CP (Selvarajan et al., 2010). In the current study, the complete genome of a new BBTV isolate from the state of Meghalaya, NE India (Supplementary Fig. S1) was characterized and compared with those of earlier reported BBTV isolates. We have studied its genetic grouping and assessed the impact of evolutionary grouping on the genomic components. We have also performed recombination analyses for better understanding of evolutionary grouping and genetic distinctiveness.

2. Materials and methods

2.1. Virus source and sequencing of the BBTV genome

Symptomatic leaf samples were collected during 2012 from infected banana plants growing naturally in Umiam, Ri-Bhoi District, Meghalaya, India on the basis of bunchy top symptom. Total DNA was extracted using a plant DNA extraction kit (Qiagen, CA). The presence of BBTV was confirmed through polymerase chain reaction (PCR) using primer pairs specific to DNA R (Vishnoi et al., 2009). Full genome characterization of the virus was performed following PCR based component amplification. The primers and PCR conditions for amplification of full-length DNA R, DNA U3, DNA S and DNA N were similar as described by Vishnoi et al. (2009) and for DNA M and DNA C, the primers and PCR conditions were according to Selvarajan et al. (2010). The PCR amplicons of six DNA components were purified from gels using GeneJET gel extraction kit (Fermentas, India). Each fragment was cloned and sequenced bi-directionally (Chromous Biotech, Bangalore, India).

2.2. Sequence analysis

The resulting sequences were assembled to get the final sequences. The identity and homology of the sequences were first evaluated using the BLASTn suit of NCBI (http://blast.ncbi.nlm.nih.gov). Finally, six DNA sequences were deposited in NCBI Sequence Database designating the complete sequence of DNA R (KC119098), DNA U3 (KC466373), DNA S (KC466374), DNA M (KC466375), DNA C (KC466376) and DNA N (KC466377) of Umiam isolate (BBTV-Umiam) from Meghalaya, India.

2.3. Phylogenetic analysis

The phylogenetic analysis of DNA R was performed to understand the genetic grouping of BBTV-Umiam. The complete nucleotide sequence of DNA R of BBTV-Umiam was compared with 87 full length sequences of BBTV DNA R available in GenBank. Two full length abaca bunchy top virus (ABTV) DNA R were used as out group member. A total of 90 sequences were aligned using ClustalW algorithm of MEGA5 (www.megasoftware.net), each beginning at the origin of replication (TATTAC). The Phylogenetic tree for DNA R was constructed on the matrices of aligned sequences with 1000 bootstrap replicates following neighbour-joining phylogeny of MEGA5 (Saitou and Nei, 1987; Tamura et al., 2011).

2.4. Comparative analysis of genomic components

Further to assess the impact of genetic grouping on each genomic component of BBTV, we have compared six components, as well as, the corresponding ORF of BBTV-Umiam with the reported BBTV isolates having only the complete genome. Out of 20 complete sequences of BBTV previously available in database, 15 are from Pacific–Indian Oceans region and rest five are representative of South-East Asian region (Supplementary Table S1). The same ABTV isolates were considered as out group members. Each component was rearranged to start with TATTAC. The pair-wise multiple alignments of nucleotide and amino acid sequences were performed using the CLUSTAL W algorithm in Meg-Align™ program of Lasergene software package (DNASTAR Inc. 7.1.0, USA) at its default settings. The phylogenetic trees for the genomic components were constructed on the matrices of aligned sequences with 1000 bootstrap replicates.

2.5. Recombination analysis

Recombination analysis was carried out using seven different methods (RDP, GENCOV, BOOTSCAN, MAXCHI, CHIMERA, SISCAN and 3SEQ) implemented in RDP4 (version 4.22; Martin et al., 2010). Each genomic component of 21 BBTV isolates (Supplementary Table S1) were aligned individually and analyzed independently for intra-component recombination. On the other hand, a separate dataset was generated including all genomic components (126) of same BBTV isolates (Supplementary Table S1) to investigate the inter-component recombination events. Recombination signals detected by at least three recombination detection methods, coupled with phylogenetic evidence of recombination, were considered as genuine recombination events.

3. Results

3.1. Genomic features of BBTV-Umiam

The genome organization of BBTV-Umiam has been determined considering Lucknow isolate of BBTV (BBTV-Lucknow, Vishnoi et al., 2009) as a standard representative. The genomic features of BBTV-Umiam are presented in Table 1. Analysis of sequence data