Genetic diversity of banana bunchy top virus isolates from China

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Summary. – Banana bunchy top virus (BBTV) (the genus Babuvirus, the family Nanoviridae) is a single-stranded circular DNA virus with a genome composed of six components designated as DNA-R, -U3, -S, -M, -C, and -N. This study analyzed the nucleotide identities of the DNA-R of 23 isolates from banana-producing provinces of China, including Guangdong, Hainan, Guangxi, and Yunnan. Results showed that the nucleotide identity of DNA-R was 72.3–100%. Phylogenetic analysis indicated that these BBTV isolates were clustered in different subgroups within the Asian group (AG). Sequence analysis of the five other components (DNA -U3, -S, -M, -C, and -N) of the five isolates from China confirmed the results established for DNA-R of these BBTV isolates. This study suggested that the variation of DNA-R from Chinese BBTV isolates was considerably higher than the variation of other AG isolates, but their genetic diversity was low.

Keywords: banana; banana bunchy top virus; genetic diversity; phylogenetic analysis

Introduction

Banana bunchy top virus (BBTV, the genus Babuvirus, the family Nanoviridae) is isometric and contains six circular single-stranded DNA genome components (designated as DNA-R, -U3, -S, -M, -C, and -N), which encode the master replication initiation protein, a protein with unknown function, capsid protein, movement protein, cell cycle link protein, and nuclear-shuttle protein, respectively (Timchenko et al., 2000). Each component is monocistronic and encodes a single open reading frame (ORF), with the exception of DNA-R (Beetham et al., 1999). Previous studies categorized BBTV isolates from the South Pacific group (SPG) and Asian group (AG) on the basis of the gene sequence identity of the DNA-R (Hu et al., 2007). Given its multicomponent characterISTICS, BBTV may undergo genetic recombination and reassortment (Hu et al., 2007; Stainton et al., 2015).

Banana (Musa spp.) is one of the important fruits in tropical and subtropical regions of China and is mainly grown in Guangdong, Guangxi, Yunnan, Fujian, and Hainan provinces. Banana bunchy top disease (BBTD), caused by BBTV, has become an important banana disease in China, the incidence of BBTD in recent years has ranged from approximately 70% to 80% in some old Chinese banana growing areas (Rao et al., 2013). Understanding the genetic diversity of BBTV is helpful to develop a reliable diagnosis and management strategies for BBTD. There are a few reported BBTV isolates in China (Feng et al., 2010; Stainton et al., 2015), most of the genetic diversity of BBTV isolates was analyzed based on DNA-R of BBTV. In this study, BBTV isolates were extensively investigated and collected from different provinces in China. The genetic diversity of these isolates was analyzed. This study performs a systematic analysis on six BBTV components of different isolates in China and comprehensively assesses the genetic diversity of DNA-R of the BBTV isolates, which will be helpful to understand the evolution of BBTV.

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Abbreviations: AG = Asian group; BBTD = banana bunchy top disease; BBTV = banana bunchy top virus; ORF = open reading frame; SPG = South Pacific group.
Materials and Methods

Samples collection and BBTV detection. Thirty-nine banana leaf samples showing typical symptoms of BBTV were collected from Guangdong, Hainan, Guangxi, and Yunnan provinces of China from 2012 to 2013, as shown in Table 1, and kept in -80°C freezer. Total DNA was extracted from banana leaves through the CTAB method (Dellaporta et al., 1983). The extracted DNA was then detected via endpoint PCR.

PCR amplification and cloning. PCR amplifications were performed using primers of Tian et al. (2004) and Feng et al. (2010) on a TaKaRa PCR Thermal Cycler under the following conditions: 98°C for 1 min; 30 cycles of 98°C for 15 s, 51–55°C for 15 s, and 68°C for 1 min; and an extension of 72°C for 10 min. The PCR products were cloned into pMD18-T (Takara, Dalian, China) in accordance with the manufacturer’s instructions, and the recombinant plasmids were sequenced.

Genetic diversity and phylogenetic analyses. Multiple alignments and pairwise nucleotide identities of DNA-R sequences were carried out through MEGA software (Tamura et al., 2013). Phylogenetic analyses were conducted using the sequence alignments through the neighbor-joining algorithm with 1000 bootstrap replications, as implemented with the MEGA software version 6.0 (Tamura et al., 2013). Five isolates were selected for further analysis to determine the genetic diversity of the BBTV isolates across all components. These isolates were selected randomly from Guangdong, Guangxi, and Hainan provinces in China. The isolates, which were used for analysis in this study, are listed in Table 1.
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Isolate Source Acc. No. Group Isolate Source Acc. No. Group

MS6_Philippines KM607666.1 AG TOS93_TO_2010 Tonga KM607721.1 SPG

1G3 Indonesia AB186924.1 AG Q570_TO_1990 Tonga KM607683.1 SPG

11S11 Indonesia AB186926.1 AG 536_TO_1993 Tonga KM607600.1 SPG

BA-1 Indonesia AB476360.1 AG TOS28 Tonga JP957636.1 SPG

520_ID_1995 Indonesia KM607593.1 AG Hawaiian USA U18077.1 SPG

Viet Nam AF416475.1 AG KP9_US_1990 USA KM607660.1 SPG

V6 Viet Nam AB113659.1 AG 527_US_1992 USA KM607599.1 SPG

Viet Nam AF416474.1 AG Egypt AF416465.1 SPG

Viet Nam AF416472.1 AG Egyptian Egypt AF102780.1 SPG

DDW* GD:Guangzhou KT215071 AG MY01 Myanmar AB252639.1 SPG

DW-1* GD:Guangzhou KT215075 AG Cameroon GQ249344.1 SPG

DW-2* GD:Guangzhou KT215076 AG Fiji AF146466.1 SPG

DN3-6* GD:Zengcheng KT215073 AG DNN* GD:Zengcheng KT215074 AG

XL-2* GX:Longan KT215084 AG HLM* HN:Lintao KT215080 AG

XP-2* GX:Pubei KT215087 AG XJ-2* GX:Nanning KT215084 AG

XSN* GX:Nanning KT215088 AG HF-2* HN:Chengmai KT215079 AG

XHD* GX:Hupe KT215083 AG YLJ-17* YN:Baoshan KT215090 AG

YLZ-3* YN:Baoshan KT215092 AG YLZ-4* YN:Baoshan KT215093 AG

YLM* YN:Linxao KT215091 AG HS-1* HN:Sanya KT215081 AG

DW-4* GD:Guangzhou KT215077 AG DW-4* (U3) GD:Guangzhou KK783438 AG

DW-4* (S) GD:Guangzhou KT779465 AG DW-4* (M) GD:Guangzhou KK779455 AG

DW-4* (C) GD:Guangzhou KK779460 AG DW-4* (N) GD:Guangzhou KK787074 AG

HF-1* HN:Chengmai KT215078 AG HF-1* (U3) HN:Chengmai KK783437 AG

HF-1* (S) HN:Chengmai KT779466 AG HF-1* (M) HN:Chengmai KK779456 AG

HF-1* (C) HN:Chengmai KT779461 AG HF-1* (N) HN:Chengmai KK787073 AG

HS-5* HN:Sanya KT215082 AG HS-5* (U3) HN:Sanya KK783436 AG

HS-5* (S) HN:Sanya KT779467 AG HS-5* (M) HN:Sanya KK779457 AG

HS-5* (C) HN:Sanya KT779462 AG HS-5* (N) HN:Sanya KK779702 AG

XP-1* GX:Pubei KT215086 AG XP-1* (U3) GX:Pubei KK783435 AG

XP-1* (S) GX:Pubei KT779468 AG XP-1* (M) GX:Pubei KK779458 AG

XP-1* (C) GX:Pubei KT779463 AG XP-1* (N) GX:Pubei KK787070 AG

XTD* GX:Nanning KT215089 AG XTD* (U3) GX:Naming KK783434 AG

XTD* (S) GX:Naming KT779469 AG XTD* (M) GX:Naming KK779459 AG

XTD* (C) GX:Naming KT779464 AG XTD* (N) GX:Naming KK787071 AG

DN3-1* GD:Zengcheng KT215072 AG

Notes: AG: Asian group. SPG: South Pacific group. GD: Guangdong province of China; GX:Guangxi province of China. YN: Yunnan province of China. HN: Hainan province of China; *: isolates in this study. (U3), (S), (M), (C), and (N) means different BBTV component, respectively, the others stand for DNA-R component of BBTV.

Results and Discussion

**BBTV detection via PCR**

PCR assays were performed with the primer set DNA1F/R (Tian et al., 2004) to detect BBTV in 39 suspected banana samples collected from Guangdong, Hainan, Guangxi, and Yunnan provinces of China. The DNA-R gene fragments of 1025 bp were generated from 23 samples. Among them 4 were from Yunnan province, 7 from Guangxi province, 5 from Hainan province, and 7 from Guangdong province. The results suggested that these 23 banana samples were infected by BBTV.

**Genetic diversity analysis of DNA-R of BBTV isolates from China**

The DNA-R components of these 23 BBTV isolates were selected for genetic diversity analysis. Results showed that
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Phylogenetic analysis of BBTV isolates based on nucleotide sequences of DNA-R components along with corresponding sequences of BBTV isolates available from GenBank

The isolates shared 72.3–100% identity at the nucleotide level. Compared among 23 BBTV isolates in this study, the nucleotide identity ranged from 86.0% to 100%, most of them had 94.2–100% nt identity, while four isolates HF-1, HF-2, HS-5, and XP-1 shared 76.4–95% nt identity with other BBTV isolates in this study, respectively. Compared with DNA-R of 53 BBTV isolates from GenBank, the results showed that the nucleotide identity ranged from 72.3% to 98.3%, most of BBTV isolates shared 82.9–92.4% nt identity, while HS-5 and XP-1 had 72.3–80.8% nt identity, HF-1 and HF-2 shared 91.2–98.3% nt identity with those other BBTV isolates. These results showed that the maximum sequence variability of DNA-R was 14% among the 23 isolates from China. This value was considerably higher than those of the isolates from the AG (Stainton et al., 2015), which is different from the previously mentioned rates of BBTV divergence (Hu et al., 2007; Stainton et al., 2015).

Phylogenetic analysis of the DNA-R of BBTV isolates from China

Phylogenetic analysis of the DNA-R components of the 23 BBTV isolates in this study together with 53 BBTV isolates available from GenBank showed that these 76 BBTV isolates were clustered into two distinctive subgroups (Fig. 1), representing AG (Subgroups 1, 2, 3, and 4) and SPG (Subgroups 5 and 6), respectively. However, the 23 BBTV isolates in this study fell into two subgroups (Subgroups 2 and 3) in AG. Subgroup 2 consisted of 8 isolates in this study, 6 from Guangdong, one from Hainan, and one from Guangxi, respectively. Subgroup 3 comprised 15 isolates, 4 from Hainan, 4 from Yunnan, 6 from Guangxi, and one from Guangdong. Our results suggested that the BBTV isolates had distinct geographical distribution (Fig. 1), which is consistent with previous results (Yu et al., 2012; Banerjee et al., 2014).

Genetic diversity of DNA-U3, -S, -M, -C, and -N of BBTV isolates from China

Isolates DW-4, HF-1, XP-1, XTD, and HS-5 were randomly selected for further analysis of the genetic diversity of DNA-U3, -S, -M, -C, and -N to determine whether or not the analysis of these components supports the results of the

Fig. 1

Phylogenetic analysis of BBTV isolates based on nucleotide sequences of DNA-R components along with corresponding sequences of BBTV isolates available from GenBank

The isolates obtained in this study are indicated by ▲ S (Table 1). The trees were constructed using the neighbor-joining method. The numbers at the nodes indicates bootstrap support (1,000 replicates). Values are shown only when the values are equal or greater than 70%.
analysis of BBTV isolates based on DNA-R. The nt identity of DNA-R of the five isolates was 87.2–99.7%, and they were located in Subgroups 2 and 3 of the AG (Fig. 1). Phylogenetic analysis based on the gene sequences of DNA-U3 showed that the five isolates grouped with the Asian isolates (Fig. 2a), in which four isolates (HS-5, XP-1, XTD, and DW-4) shared 89.5–99.5% nt identity and grouped with Taiwan isolates DQ826391 and DQ826392 (Fig. 2a), while HF-1 grouped with Hainan isolates GU559705 and GU559706, and shared 77.8–85.4% nt identity with other four isolates. Sequence analysis based on DNA-S showed that the nt identity among these isolates was 92.7–100%, the phylogenetic analysis showed that these five isolates were clustered in the AG in two subgroups (Fig. 2b). According to DNA-M sequence analysis, the five isolates were clustered in the same subgroup into three branches (Fig. 2c), the nt identity among these 5

The GenBank Acc. Nos. in this study are listed in Table 1. The trees were constructed using the neighbor-joining method. The numbers at the nodes indicate bootstrap support (1,000 replicates).
isolates was 90.3–99.7%. Analysis results of DNA-C showed that the five isolates were clustered in the same subgroup (Fig. 2d), the nt identity among these isolates was 97.2–100%, and the nt identity of XP-1 and HS-5 was 100%. Sequence analysis of DNA-N showed that the five isolates were clustered in the same subgroup (Fig. 2e), the nt identity among these isolates ranged from 93.4% to 99.9%.

These results confirmed the sequence and phylogenetic analyses of BBTV DNA-R, which supported the geographical structuring of BBTV isolates (Hu et al., 2007; Yu et al., 2012). However, DNA-U3 was more variable (22.2%) than other components of BBTV (2.8–14%), which was caused by different evolutionary pressures on each component and/or DNA component recombination in the genome of BBTV isolates (Hu et al., 2007; Hyder et al., 2011). Noticeably, DNA-U3 in this study was detected the absence of an additional TATA box or a small ORF, this finding is opposite to previous reports (Yu et al., 2012), thereby emphasizing the additional components of DNA-U3. Significance of this observation is not known and warrants further investigation.

Although the genetic diversity of the BBTV isolates was reported (Hu et al., 2007; Banerjee et al., 2014), it was mostly assessed based on DNA-R or DNA-U3 of BBTV. The current study presented that the sequence data on the six components of the five BBTV isolates from China will be useful in future studies of these BBTV components.

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References


