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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 though 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.

Table 1. Details of BBTV isolates used in analyses obtained from GenBank and in this study

Isolate	Source	Acc. No.	Group	Isolate	Source	Acc. No.	Group
NSP	China	AF238875.1	AG	Etawah	India	DQ656119.1	SPG
Guangdong-1	China	AF246123.1	AG		India	EU140342.1	SPG
NS	China	AF238874.1	AG		India	AF416470.1	SPG
C4	China	U97525.1	AG	Bihar	India	FJ605506.1	SPG
Haikou-4	China	HQ378190.1	AG	Bangalore-GKVK	India	JN243751.1	SPG
DanZhouHD	China	GU559706.1	AG	Andaman	India	EU531473.1	SPG
Haikou-4	China	HQ378192.1	AG	Lucknow	India	DQ256267.1	SPG
DanZhou	China	GU559705.1	AG		India	AY845437.1	SPG
Haikou-4	China	HQ378191.1	AG		India	EU140341.1	SPG
Haikou-4	China	HQ378193.1	AG	Bihar	India	FJ605508.1	SPG
Haikou-4	China	HQ378194.1	AG	Lucknow	India	EU402601.2	SPG
	China:Taiwan	DQ826391.1	AG	Bihar	India	FJ609644.1	SPG
	China:Taiwan	DQ826394.1	AG	Bihar	India	FJ609642.1	SPG
V-1	China:Taiwan	EF095165.1	AG	Bihar	India	FJ609643.1	SPG
	China:Taiwan	DQ826395.1	AG	Bangalore-GKVK	India	JN243752.1	SPG
V-1	China:Taiwan	EF095166.1	AG	Bihar	India	FJ605507.1	SPG
	China:Taiwan	DQ826392.1	AG	Bangalore-GKVK	India	JN243753.1	SPG
V-1	China:Taiwan	EF095163.1	AG	Bangalore-GKVK	India	JN243754.1	SPG
	China:Taiwan	DQ826393.1	AG	Q553_LK_1995	Sri Lanka	KM607680.1	SPG
V-1	China:Taiwan	EF095164.1	AG	KP5_LK_2003	Sri Lanka	KM607656.1	SPG
	China:Taiwan	DQ826396.1	AG	Kandy	Sri Lanka	JN250593.1	SPG
V-1	China:Taiwan	EF095162.1	AG	Kandy	Sri Lanka	JN250594.1	SPG
	China:Taiwan	DQ826390.1	AG	Kandy	Sri Lanka	JN250596.1	SPG
V-1 clone b	China:Taiwan	EF095161.1	AG	Kandy	Sri Lanka	JN250597.1	SPG
TW3	China:Taiwan	EU366169.1	AG	Kandy	Sri Lanka	JN250598.1	SPG
Q623_TW_1996	China:Taiwan	KM607684.1	AG	Kandy	Sri Lanka	JN250595.1	SPG
JY3	Japan	AB108457.1	AG	clone mrep 2	Pakistan	AM418534.1	SPG
JK3	Japan	AB108453.1	AG	TJ1	Pakistan	AY996562.2	SPG
JM5	Japan	AB108454.1	AG	TJ3	Pakistan	JX170762.1	SPG
JN4	Japan	AB108452.1	AG	HD2	Pakistan	FJ859734.1	SPG
bP5	Philippines	AB189067.1	AG	clone mrep 3	Pakistan	AM418536.1	SPG
	Philippines	AF416469.1	AG	clone mrep jav	Pakistan	AM418538.1	SPG
aP34	Philippines	AB250954.1	AG		Tonga	AF416467.1	SPG

Table 1 (continued)

Isolate	Source	Acc. No.	Group	Isolate	Source	Acc. No.	Group
MS6_PH_2008	Philippines	KM607666.1	AG	TOS93_TO_2010	Tonga	KM607721.1	SPG
IG33	Indonesia	AB186924.1	AG	Q570_TO_1990	Tonga	KM607683.1	SPG
IJs11	Indonesia	AB186926.1	AG	536_TO_1993	Tonga	KM607600.1	SPG
BA-1	Indonesia	AB847630.1	AG	TOS28	Tonga	JF957636.1	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:Dongguang	KT215071	AG	MY01	Myanmar	AB252639.1	SPG
DW-1*	GD:Guangzhou	KT215075	AG		Cameroon	GQ249344.1	SPG
DW-2*	GD:Guangzhou	KT215076	AG		Fiji	AF416466.1	SPG
DN3-6*	GD:Zengcheng	KT215073	AG	DNN*	GD:Zengcheng	KT215074	AG
XL-2*	GX:Longan	KT215084	AG	HLM*	HN:Lingao	KT215080	AG
XP-2*	GX:Pubei	KT215087	AG	XJ-2*	GX:Naning	KT215084	AG
XSN*	GX:Naning	KT215088	AG	HF-2*	HN:Chengmai	KT215079	AG
XHD*	GX:Hepu	KT215083	AG	YLJ-17*	YN:Baoshan	KT215090	AG
YLZ-3*	YN:Baoshan	KT215092	AG	YLZ-4*	YN:Baoshan	KT215093	AG
YLM*	YN:Linpao	KT215091	AG	HS-1*	HN:Sanya	KT215081	AG
DW-4*	GD:Guangzhou	KT215077	AG	DW-4* (U3)	GD:Guangzhou	KX783438	AG
DW-4* (S)	GD:Guangzhou	KX779465	AG	DW-4* (M)	GD:Guangzhou	KX779455	AG
DW-4* (C)	GD:Guangzhou	KX779460	AG	DW-4* (N)	GD:Guangzhou	KX787074	AG
HF-1*	HN:Chengmai	KT215078	AG	HF-1* (U3)	HN:Chengmai	KX783437	AG
HF-1* (S)	HN:Chengmai	KX779466	AG	HF-1* (M)	HN:Chengmai	KX779456	AG
HF-1* (C)	HN:Chengmai	KX779461	AG	HF-1* (N)	HN:Chengmai	KX787073	AG
HS-5*	HN:Sanya	KT215082	AG	HS-5* (U3)	HN:Sanya	KX783436	AG
HS-5* (S)	HN:Sanya	KX779467	AG	HS-5* (M)	HN:Sanya	KX779457	AG
HS-5* (C)	HN:Sanya	KX779462	AG	HS-5* (N)	HN:Sanya	KX787072	AG
XP-1*	GX:Pubei	KT215086	AG	XP-1* (U3)	GX:Pubei	KX783435	AG
XP-1* (S)	GX:Pubei	KX779468	AG	XP-1* (M)	GX:Pubei	KX779458	AG
XP-1* (C)	GX:Pubei	KX779463	AG	XP-1* (N)	GX:Pubei	KX787070	AG
XTD*	GX:Naning	KT215089	AG	XTD* (U3)	GX:Naning	KX783434	AG
XTD* (S)	GX:Naning	KX779469	AG	XTD* (M)	GX:Naning	KX779459	AG
XTD* (C)	GX:Naning	KX779464	AG	XTD* (N)	GX:Naning	KX787071	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.

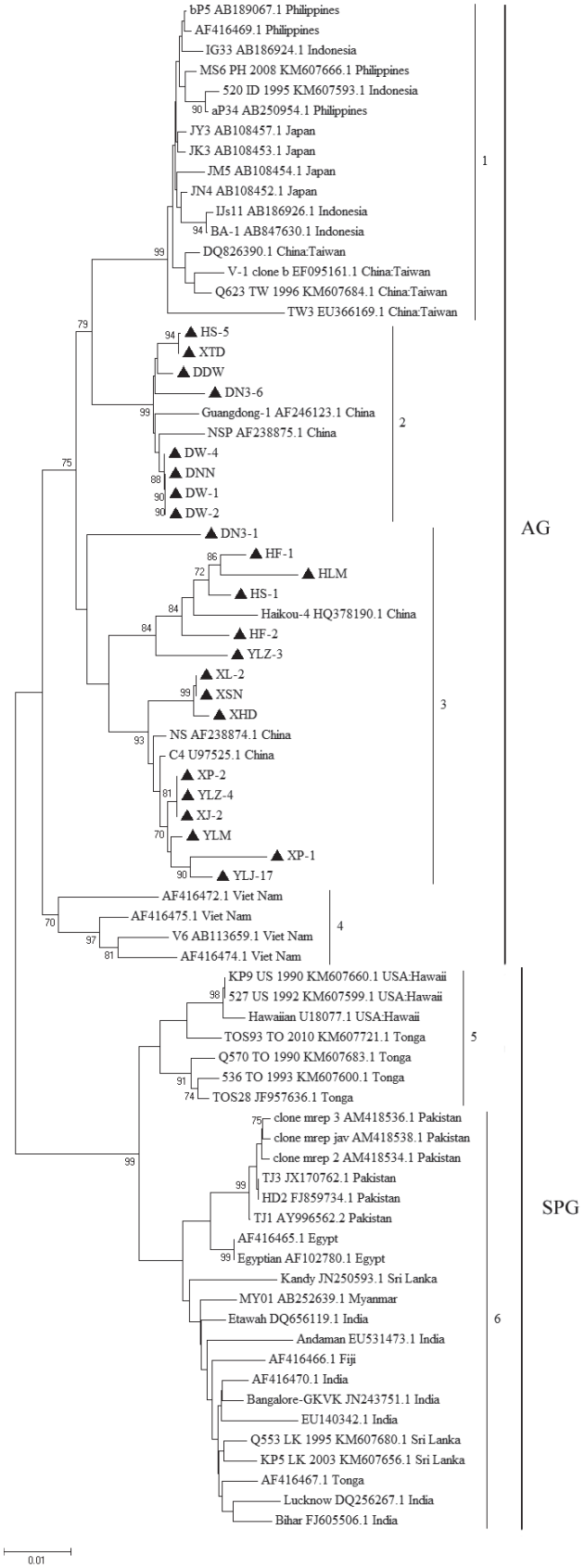
Results and Discussion

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



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 "▲" (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%.

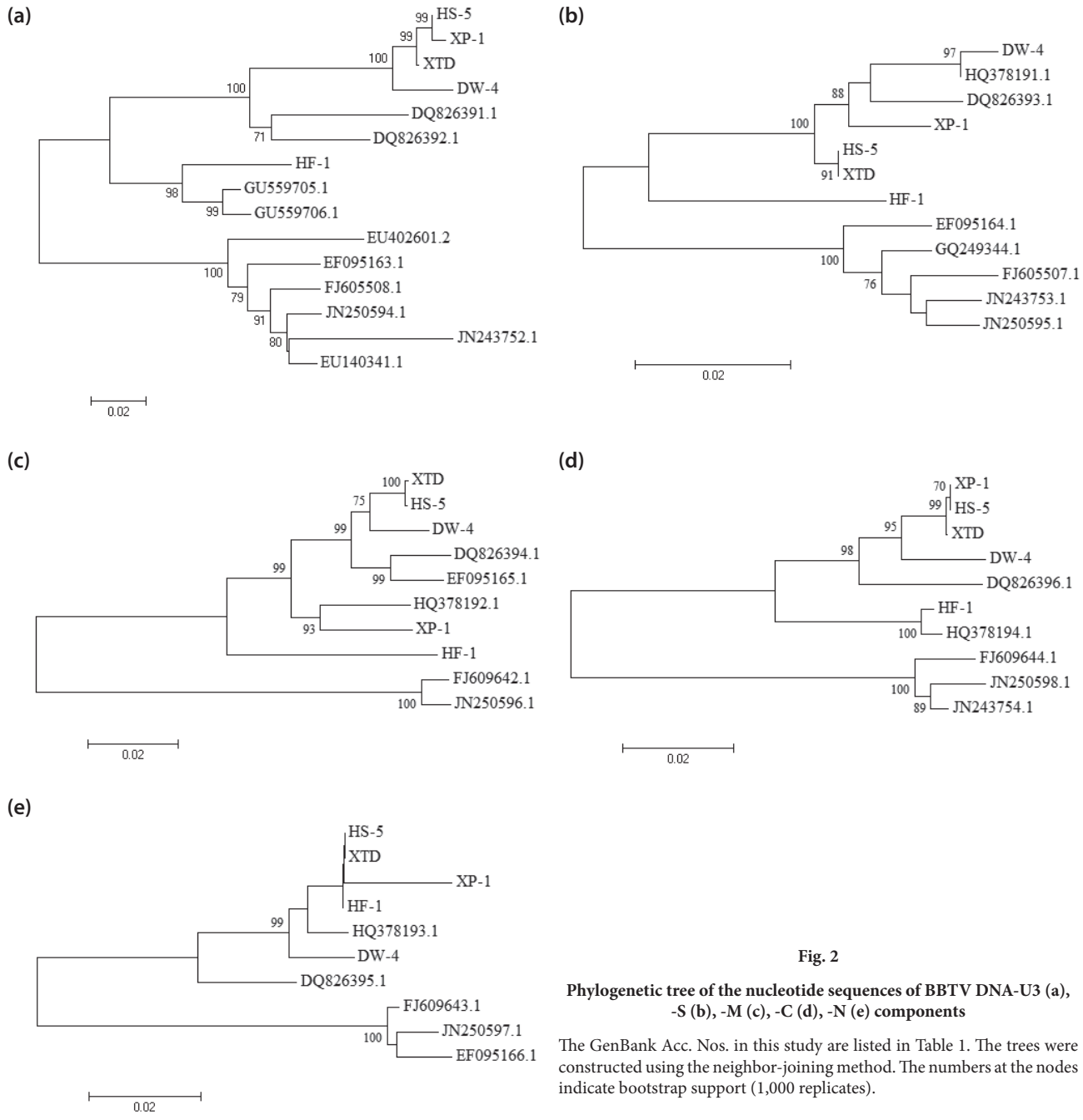


Fig. 2

Phylogenetic tree of the nucleotide sequences of BBTV DNA-U3 (a), -S (b), -M (c), -C (d), -N (e) components

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).

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

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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