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Molecular Characterization of *Banana bunchy top virus* and *Cucumber mosaic virus* from Abaca (*Musa textilis* Nee)

By

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Summary : Abaca (*Musa textilis* Nee), a fiber crop, is considered one of principal industrial-crops in the Philippines. This study was conducted to clarify the occurrence and the molecular characterization of *Banana bunchy top virus* (BBTV), *Cucumber mosaic virus* (CMV), and *Banana bract mosaic virus* (BBrMV) in abaca and banana plants collected in Luzon and Negros islands, the Philippines. In abaca, we detected BBrMV from 34, BBTV from 12, and CMV from 6 out of 37 samples. In banana, we confirmed BBTV from 19, BBrMV from 14, and CMV from 3 out of 25 samples. These results revealed that BBrMV for abaca, BBTV and BBrMV for banana were major viruses in the studied area, although abaca bunchy top was recognized as the most serious disease economically. Several abaca varieties were evaluated for their resistance against bunchy top disease and found some hybrids to show resistant reaction. The putative causal virus of abaca bunchy top disease was partially sequenced, and the 3 genes of the replication initiation protein, the coat protein, and the movement protein were compared with those of related viruses previously reported. As the virus isolated from abaca plants showed more than 99% of homologies with BBTVs isolated from banana plants by comparison of the putative amino acid sequences of the each genes, both isolates were shown as identical in molecular characterization. Moreover, this study showed the first detection of CMV from abaca by ELISA and RT-PCR. Based on the sequence analysis of the coat protein gene, one abaca isolate of CMV was classified as subgroup IB.

Key words : abaca, banana, bunchy top disease, *Banana bunchy top virus,* Philippines

Introduction

Abaca (*Musa textilis* Nee), often known as Manila hemp, is endemic to the Philippines and cultivated in some parts of the country. It has been the source of high quality fibers for cordage products such as ropes and binders, pulp and paper manufactures, and others. In Japan, the fiber of abaca is used as a material of a ten thousand yen bill and ropes for tug-of-war. According to the Fiber Industry Development Agency (FIDA) in the Philippines (FIDA, 2005 : http : //fida.da.gov.ph), since 1995 through 2004, abaca production averaged 68,112 Mt, which is equivalent to an increase of 1.5% annually. In 2004, the total production of abaca fiber reached 72,891 Mt, an increase of 4.4% over the 2003 level. In the same year, a total of 77,526 abaca farmers have been cultivating a total of 127,258 ha. By the end of 2004, a total of 8,252 ha of new abaca farms were established in abaca producing areas of the Philippines and each farmer has about 2 ha on average. There has been no steady increase in production of abaca through the years due to several factors, e.g. dampening foreign

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demand due to global recession, lack of healthy planting materials, and infestation of pests and diseases more specifically abaca bunchy top and mosaic. Although the world production of abaca has declined to more than half of that in the first half of the 20th century when the cultivation was very active, approximately 69% of the abaca world production is still from the Philippines in the year of 2005 (FAOSTAT, 2006 : http : //faostat.fao.org/).

As abaca belongs to the same genus *Musa* with banana and plantain (*Musa* spp.), it will be significant to study viruses occurring on abaca and banana for the protection of their virus diseases. Bunchy top disease in abaca was first observed in Silang, Cavite, the Philippines, as early as 1915 but became serious from 1923 up to this date. Due to this disease, many plantations in Cavite and Laguna, the Philippines were abandoned by farmers because abaca became unproductive. The disease spread to other parts of the country specifically Luzon and Bicol regions where many farmers plant abaca as a source of cash and for fiber craft industry.

The symptoms of abaca bunchy top disease (ABTD) and banana bunchy top disease (BBTD) in banana are very similar in terms of stunting and narrowing of the leaves. Most of the commercial banana cultivars in the Philippines like Lakatan, Latundan, and Saba are susceptible to bunchy top disease. However, the first official report of ABTD[23] was 35 years earlier than that of BBTD[6] in the Philippines.

In "Banana Diseases" by Wardlaw[34], the description by Ocfemia (1927) was cited that some banana cultivars, growing in the neighboring area of abaca seriously affected with ABTD, were apparently not susceptible, and Monsalad (1933) reported that the ABTD was formerly thought to be quite distinct from BBTD and caused by a different virus. Whereas, Magee[20] succeeded in aphid transmission of the disease from abaca to banana and from banana to abaca by banana aphid, *Pentalonia nigronervosa* Coq., and concluded that there may be at least three active strains or types of the causal virus in different Philippine islands. In "Disease of Banana, Abaca and Enset"[16], the description by Thomas and Bajet (1993, unpublished) was cited that *Banana bunchy top virus* (BBTV) was detected in a sample affected by ABTD by ELISA in the Philippines.

Based on aphid transmission and serological properties of the causal virus, the pathogen of ABTD which is referred to as Abaca bunchy top virus (ABTV) is considered to be the synonym of BBTV, the pathogen of banana bunchy top disease. However, direct comparison of these two viruses from abaca and banana plants by molecular method has not been studied yet.

The objectives of this study are to detect the viruses from abaca and banana plants in the Philippines to learn their major viruses, evaluate the resistance of abaca varieties against ABTD, detect the putative causal virus of ABTD and characterize it to study identity with BBTV. A part of characteristics of *Cucumber mosaic virus* (CMV) from abaca was also studied because the detection of CMV from abaca has not been carried out on serological and molecular basis.

Materials and Methods

Plant materials

From 1999 to 2003, 37 samples of abaca and 25 samples of banana showing symptoms of viral disease such as bunchy top, mosaic, and distortion were collected in Luzon and Negros islands, the Philippines. These materials were imported to Japan with the permission of the Ministry of Agriculture, Forestry and Fisheries, Japan (15-Yokohama PQ 361, 2003).

Virus detection by ELISA

To detect 3 major *Musa* viruses, BBTV, CMV, and *Banana bract mosaic virus* (BBrMV), ELISA kits (Agdia, USA) were used according to the manufacturer's instructions. We evaluated the sample which showed 3 times higher absorbance value at A_{405nm} than that of the healthy sample as positive reaction to the virus.

Evaluation of resistance of local abaca varieties to abaca bunchy top disease

Local abaca varieties and hybrids in the Abaca Germplasm Collection of the Institute of Plant Breeding, the Philippines were evaluated for resistance to bunchy top disease. For field evaluation, about 13 varieties/hybrids were evaluated in Albay, a part of the Bicol region where almost all abaca varieties are popularly grown. ABTD is the most serious disease of abaca in the Bicol region. Hence, there is enough disease pressure that warrants field evaluation. Percent incidence, number of plants showing ABTD symptoms over the total number of plants, was taken at flowering and harvesting stages. For screen house evaluation, abaca was grown from corms or seeds in plastic pots. After two months, each plant was inoculated with viruliferous aphids, *Pentalonia nigronervosa*. Evaluation of the reaction of the inoculated plants was determined after one month using the rating scale/disease index. Five grades of Reaction Class (RC) from 1 (none to slight bunchy top symptoms) to 5 (very severe symptoms-leaves badly deformed and almost bladeless) with responded class value (CV) of 10, 30, 50, 70 and 100,

respectively. Then Disease Index (=number of seedlings in RC×CV/number of inoculated seedlings) was calculated. Resistance was judged by Disease Index of 0 as highly resistant, 1 as resistant, 2 as moderately resistant, 3 as moderately susceptible, 4 as susceptible and 5 as highly susceptible.

Polymerase chain reaction (PCR)

To amplify the genome of BBTV by PCR, total DNA was extracted from plant midribs (0.1 g) using a PhytoPure DNA extraction kit (Nucleon, UK). Amplification of full length of each of three BBTV components, DNA-R, DNA-S, and DNA-M, were carried out using TaKaRa Ex Taq™ (TaKaRa, Japan) and the primers shown in Table 1. The reaction steps were as follows : 94℃ 4 min, 30 cycles of 94℃ 1 min, 45 to 61℃ 1 min, 72℃ 2 min, and 72℃ 10 min.

Reverse transcriptase-PCR (RT-PCR)

To amplify the genome of CMV by RT-PCR, total RNA was extracted from plant leaves (0.1 g) using 1% SDS following phenol/chloroform extraction[27]. The cDNA synthesis was carried out using a reverse primer, 10R (5' CGC CCT GCA GTG GTC TCC TTT TGG A 3', KAWAGUCHI and NATSUAKI, personal communication), and the First-Strand cDNA Synthesis Kit (Amersham Biosciences, UK) at 37℃ for 1 hour. Then, the coat protein gene of CMV was amplified by PCR using TaKaRa Ex Taq™ (TaKaRa, Japan) and *Cucumovirus* universal primers[7] (Table 1).

Cloning and sequencing analysis

The amplified products were fractionated on 2% agarose gel in Tris-Acetate-EDTA (TAE) buffer and stained with ethidium bromide. Then each target band, approximately 1 kbp, was recovered using a QIAquick™ Gel Extraction kit (QIAGEN, Germany). After the bands were ligated into the pGEM-T Vector (Promega, USA) and transformed into *E. coli* DH5α (Life Technologies Inc., USA), appropriate clones were selected following mini-preparation using LaboPass™ Mini Plasmid DNA Purification Kit (Hokkaido System Science, Japan). We sequenced at least 3 clones using the ABI PRISM 377 DNA Sequencer with an ABI Prism BigDye® Terminator Cycle Sequencing Kit (Applied Biosystems, USA).

Nucleotide and amino acid sequence alignments, and homology analysis were performed using Assembly LIGN 1.0.9c (Accelrys, USA), CLUSTAL W package[31] with Mac Vector 6.5.3 (Accelrys, USA). Phylogenetic analysis was carried out by using PAUP* 4.0 beta version[30]. Comparative sequences of BBTV DNA-R, BBTV DNA-S, BBTV DNA-M, and the coat protein gene of CMV were obtained from DDBJ/EMBL/GenBank (Table 2 and 3).

The nucleotide sequence data reported in this paper appeared in the DDBJ/EMBL/GenBank databases under accession numbers AB250953-AB250962.

Results and Discussion

Serological detection of BBTV, CMV, and BBrMV

We detected the major 3 *Musa* viruses, BBTV, CMV, and BBrMV, from 37 abaca and 25 banana samples collected in the Philippines by ELISA (Table 4). In abaca, BBrMV was detected from 34 samples, and 12 samples showed positive reaction to anti-BBTV. Moreover, 6 abaca samples reacted to anti-CMV antibody. In banana, BBTV, BBrMV, and CMV were detected from 19, 14, and 3 samples, respectively. These results revealed that BBrMV for abaca, BBTV and BBrMV for banana were their major viruses in the studied area. Judging by symptoms, however, bunchy top disease on abaca seemed the most serious and devastative in fiber production due to its severe bunchy top symptom, while the symptom caused by BBrMV was not often conspicuous.

Evaluation of resistance of local abaca varieties to abaca bunchy top disease

Percent incidence of ABTD on local cultivars and

Table 1 Sequences of primers used in this study

Target virus	Target region	Primer	Primer sequence	Reference
BBTV	DNA-R	F3	5' GGA AGA AGC CTC TCA TCT GCT TCA GAG ARC 3'	Karan et al. (1994) [17]
		FPCR4	5' TTC CCA GGC GCA CAC CTT GAG AAA CGA AAG 3'	
	DNA-S	CBT3F.PRI	5' GGT ATT TCG GAT TGA GCC TAC 3'	Wanitchakorn et al. (2000) [33]
		CBT3R.PRI	5' TTG ACG GTG TTT TCA GGA ACC 3'	
	DNA-M	J02	5' CTT CGA GGC GAA GCA AAC CA 3'	This study
		G01	5' CCG GAC GTC AAA TGT TTA TTC 3'	
Cucumovirus	coat protein	CPTALL-5	5' YAS YTT TDR GGT TCA ATT CC 3'	Choi et al. (1999) [7]
		CPTALL-3	5' GAC TGA CCA TTT TAG CCG 3'	

Table 2 BBTVs and the ralated species used in DNA analyses

Species	Isolate	Host	Country of origin	Accession no.			Reference or source
				DNA-R	DNA-S	DNA-M	
BBTV	aP32	Abaca	Philippines	AB250953	AB250956	AB250959	This study
	aP34	Abaca	Philippines	AB250954	AB250957	AB250960	This study
Asian group	bP5	Banana	Philippines	AB189067	AB189068	-	Furuya et al. (2005) [10]
	bP26	Banana	Philippines	AB250955	AB250958	AB250961	This study
	Ph	Banana	Philippines	AF416469	AF148068	-	Karan et al. (1994) [17], Wanitchakorn et al. (2000) [33]
	JN4	Banana	Japan	AB108452	AB108449	-	Furuya et al. (2005) [10]
	JK3	Banana	Japan	AB108453	AB108450	-	Furuya et al. (2005) [10]
	JM5	Banana	Japan	AB108454	-	-	Furuya et al. (2005) [10]
	JM6	Banana	Japan	AB108455	-	-	Furuya et al. (2005) [10]
	JY1	Banana	Japan	AB108456	AB108451	-	Furuya et al. (2005) [10]
	JY3	Banana	Japan	AB108457	-	-	Furuya et al. (2005) [10]
	JY7	Banana	Japan	AB108458	-	-	Furuya et al. (2005) [10]
	IG33	Banana	Indonesia	AB186924	AB186927	-	Furuya et al. (2004) [11]
	IG64	Banana	Indonesia	AB186925	AB186928	-	Furuya et al. (2004) [11]
	IJs11	Banana	Indonesia	AB186926	AB186929	-	Furuya et al. (2004) [11]
	Tw	Banana	Taiwan	AF416468	AF148942	-	Karan et al. (1994) [17], Wanitchakorn et al. (2000) [33]
	C4	Banana	China	-	-	U97527	
	C-Z	Banana	China	AF110266	AF330706	AF349568	
	C-NS	Banana	China	AF238874	AF238876	-	He et al. (2000) [14]
	C-NSP	Banana	China	AF238875	AF238877	-	He et al. (2000) [14]
	V6	Banana	Vietnam	AF113659	AF113661	-	Furuya et al. (2005) [10]
	V14	Banana	Vietnam	AF113660	AF113662	-	Furuya et al. (2005) [10]
	SL	Banana	Vietnam	AF416472	-	-	Bell et al. (2002) [1]
	DBP	Banana	Vietnam	AF416473	-	-	Bell et al. (2002) [1]
	BN	Banana	Vietnam	AF416474	-	-	Bell et al. (2002) [1]
	Hue	Banana	Vietnam	AF416475	-	-	Bell et al. (2002) [1]
	BMT	Banana	Vietnam	AF416476	-	-	Bell et al. (2002) [1]
	DN	Banana	Vietnam	AF416477	-	-	Bell et al. (2002) [1]
	HCM	Banana	Vietnam	AF416478	-	-	Bell et al. (2002) [1]
	YB	Banana	Vietnam	AF416479	-	-	Bell et al. (2002) [1]
South Pacific group	Au	Banana	Australia	S56276	L41574	NC_003474	Harding et al. (1991) [12], Burns et al. (1995) [4]
	Fj	Banana	Fiji	AF416466	AF148944	-	Karan et al. (1994) [17], Wanitchakorn et al. (2000) [33]
	Tn	Banana	Tonga	AF416467	-	-	Karan et al. (1994) [17]
	Ind	Banana	India	AF416470	-	-	Karan et al. (1994) [17]
	Eg	Banana	Egypt	AF416465	-	AF102783	Karan et al. (1994) [17]
	Hw	Banana	USA	U18077	-	-	Xie and Hu (1995) [35]
	Br	Banana	Burundi	-	AF148943	-	Wanitchakorn et al. (2000) [33]
SCSV	-	-	-	AJ290434	U16734	NC_003813	Timchenko et al. (2000) [32], Boevink et al. (1995) [3]
MDV	-	-	-	AB027511	AB009046	AB000927	Sano et al. (1998) [28]
FBNYV	-	-	-	Y11405	Y11408	AF159705	Katul et al. (1997) [19], Franz et al. (1999) [9]

Table 3 CMVs used in the coat protein gene analyses

Species	Isolate	Host	Country of origin	Accession no.	Reference or source
CMV	aP81	Abaca	Philippines	AB250962	This study
Subgroup I	Hawaii	Banana	USA	U31219	Hu et al. (1995) [15]
	Oahu	Banana	USA	U31220	Hu et al. (1995) [15]
	Ix	Tomato	Philippines	U20219	McGarvey et al. (1995) [21]
	C	-	-	D00462	Quemada et al. (1989) [26]
	FC	-	-	D10544	Shintaku (1991) [29]
	FNY	Musk melon	USA	D10538	Owen et al. (1990) [25]
	As	-	Korea	X77855	
	O	-	Japan	D00385	Hayakawa et al. (1989) [13]
	PR50	-	-	M98501	
	Y	Tobacco	Japan	D12499	Nitta et al. (1988) [22]
	China	-	China	X65017	
Subgroup II	Kin	-	-	Z12818	Boccard and Baulcombe (1993) [2]
	TRK-7	-	Hungary	L15336	
	WL	-	-	D00463	Quemada et al. (1989) [26]
PSV	J	-	Japan	D00668	Karasawa et al. (1991) [18]
TAV	B	-	-	S72468	O'Reilly et al. (1994) [24]

Table 4 ELISA detection of 3 *Musa* viruses from abaca and banana collected in the Philippines

	BBTV	BBTV+CMV	BBTV+BBrMV	BBTV+CMV+BBrMV	CMV	CMV+BBrMV	BBrMV	not detected
Abaca	2 / 37	0 / 37	8 / 37	2 / 37	0 / 37	4 / 37	20 / 37	1 / 37
Banana	7 / 25	0 / 25	11 / 25	1 / 25	1 / 25	1 / 25	1 / 25	3 / 25

hybrids in the field evaluation ranging from 0 to 100% was shown in Table 5. The local cultivars had higher percent incidence as compared to the hybrids. As for seven hybrids, they were developed at the Institute of Plant Breeding, UP Los Baños, and IPB 84–20×05 series were crossed between Itolaus 39, a known resistant variety, and Magsarapong, a known susceptible but high yielding variety. Of the seven hybrids, only one (Batang×GAES #2) showed bunchy top while the rest showed no symptom.

Of the 58 collections evaluated in screen house experiment, 24 varieties/hybrids (Pacol×CES 111–2, Pacol 62–1, Tetraploid 2, Musa agutay, Sogmad Pula, IPB 84–33×20–1, IPB 84–20×05–3, IPB 84–20×05–4, IPB 84–20×05–7, Inosa 12, Cantong, Soglin, Soglaguis Sogmin, Amokid, Daganon, Libuntanay, Lagwis, Bisaya 01, Kadaohan, Libutanay, Kalinawan Linlay, Sogmad Pula×Languis) were resistant, 6 (Sogmad, Baunan, Layahon, Lunhan, IPB 84–20×05–8, Batang×GAES #2) moderately resistant, 9 (Igit, Agatayon, Sogmad Pula, Agutay, Wild 4, Itolaus 39×Magsarapong, Tetraploid #1×Itolaus 39, Pacol×CES 2, Maguindanao×Pacol) moderately susceptible and 19 (Lunjan, Damulon, Maguindanao Black, Maguindanao White, Tinawagan Puti, Tangongon, Tinawagan Pula, San Bagui, Bongotsanon, Dehayop, Bongal-D, Danganon, Linminlay, Languis, Sa-ahon 01, Bontang, Banguisan, Luminlay, Hinagikhik) susceptible to abaca bunchy top disease. Although there are many varieties found resistant under screen house condition, they are not popularly grown in abaca growing parts of the country due to low yield and undesirable fiber characteristics. However, the most popular ones like Tinawagan Puti, Tinawagan Pula and Tangongon were revealed to be susceptible to the disease. After the field evaluation, the resistant varieties shown in this study could be used in the improvement of popular and high yielding varieties.

Table 5 Incidence of abaca bunchy top disease in the field evaluation

No.	Variety / Hybrid	Percent Incidence
1	Tinawagan Pula	100
2	Malaniceron	67
3	Baunan 1	61
4	Baunan 2	38
5	AP 2-1-2	36
6	AP 2-1-1	8
7	Batang x GAES#2	20
8	IPB 84-33 x 20-1	0
9	IPB 84-20 x 05-3	0
10	IPB 84-20 x 05-4	0
11	IPB 84-20 x 05-7	0
12	IPB 84-20 x 05-8	0
13	Sogmad Pula x Languis	0

Molecular detection and characterization of BBTV isolated from abaca

Two abaca samples, aP32 and aP34, showing bunchy top symptoms and reacted to anti-BBTV antibody, were applied to molecular detection of BBTV by PCR. Three sets of BBTV specific primers (Table 1) for 3 major components, DNA-R, DNA-S, and DNA-M, could amplify the each target DNAs from the abaca samples (data not shown).

PCR-amplified DNA fragments from these 2 abaca samples, aP32 and aP34, were sequenced after cloning, and compared with BBTV isolates from banana plants (hereafter BBTV banana isolates) previously reported in various parts of the world and the related species in the family, *Nanoviridae*. Each 3 major components of PCR-amplified DNA fragments from the abaca plants had the same genome structures with BBTV banana isolates. In the comparison of the putative amino acid

Table 6 Sequence homologies (%) of DNA-R, and 3 ORFs (DNA-R : master-rep protein, DNA-S : coat protein, DNA-M : movement protein) between abaca isolates and among abaca and banana isolates

	DNA-R:				DNA-S:		DNA-M:	
	Full- length	CR-M	Master-Rep protein		Coat protein		Movement protein	
	nt	nt	nt	aa	nt	aa	nt	aa
Between the abaca isolates from the Philippines	99	95	99	99	100	100	100	100
Among the abaca and banana isolates from the Philippines	99	95-100	99	99-100	99-100	99-100	99-100	100
Between the abaca isolates and the Asian group	92-99	89-100	92-99	94-100	94-99	94-100	95-99	87-100
Between the abaca isolates and the South Pacific group	89-90	62-67	91-92	93-95	94	98	84-89	79-93
Between the abaca isolates and SCSV	-	-	-	55-56	-	20	-	16
MDV	-	-	-	54-55	-	20	-	17
FBNYV	-	-	-	55	-	18	-	16

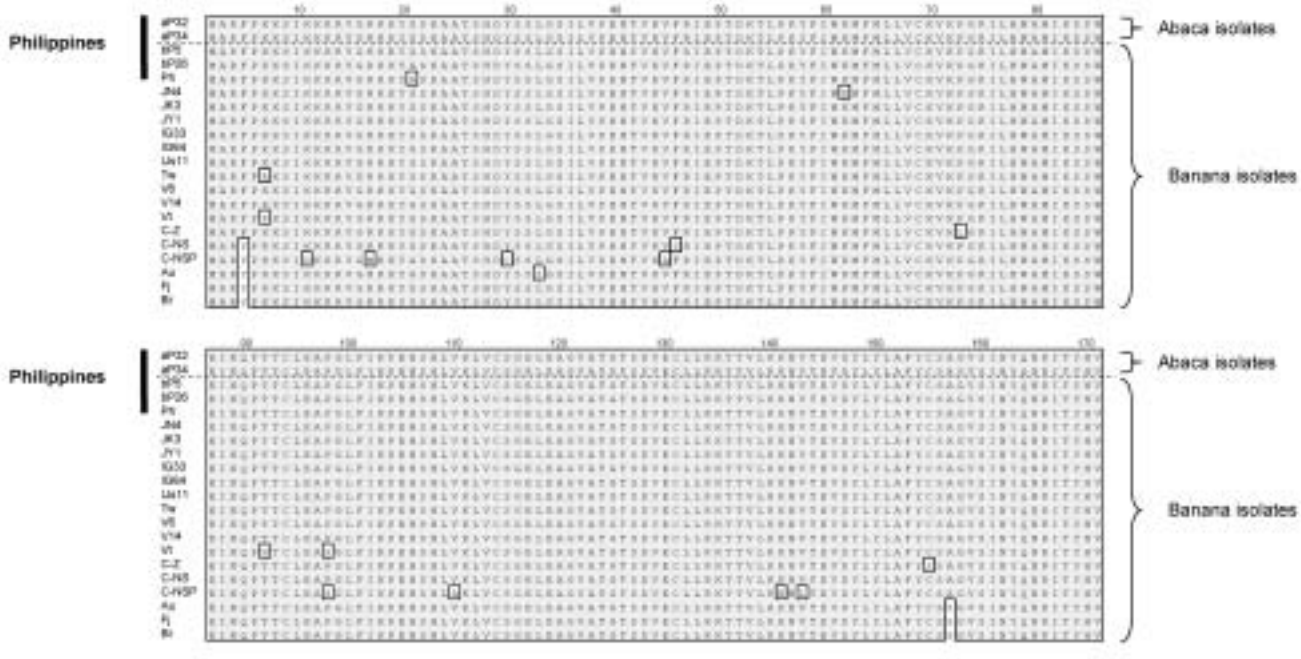

Fig. 1 The putative amino acid sequence comparison of the coat protein gene of BBTVs isolated from abaca and banana.

sequences of the coat protein gene among BBTV isolates from the Philippines, 2 abaca isolates (hereafter BBTV abaca isolates, BBTV-aP32 and BBTV-aP34) completely corresponded with 2 BBTV banana isolates, BBTV-bP26 and BBTV-bP5, while 1 banana isolate, BBTV-Ph, had one substitution of alanine from glycine at position 20 (Fig. 1).

The homology analysis of 3 major ORFs (Table 5), the replication initiation protein gene coded in DNA-R, the coat protein gene coded in DNA-S, and the movement protein gene coded in DNA-M, showed that BBTV-aP32 and BBTV-aP34 had very high homologies (99–100%) with those of all 3 BBTV banana isolates collected in the Philippines.

BBTVs isolated from bananas in the Philippines are grouped in the Asian group along with BBTVs from Indonesia and Japan[10,17]. In the major common region (CR-M) of DNA-R, the nucleotide sequences of 2 BBTV abaca isolates indicated apparently higher homologies with the Asian group (89–100%) than with the South Pacific group (62–67%).

In the Philippines, abaca bunchy top disease (ABTD) was first reported in 1926 followed by the first report of banana bunchy top disease (BBTD) 35 years later. Since then identity of the pathogens of ABTD and BBTD has been a long-time controversial issue. The pathogen of ABTD, often described as Abaca bunchy top virus (ABTV), and the pathogen of BBTD, *Banana bunchy top virus* (BBTV) are known to share similar characters such as bunchy top symptom, host range which is mainly limited to *Musa* spp., banana aphid transmission and reaction to anti-BBTV antibody. At present BBTV is officially recognized as one virus species in the genus *Babuvirus* by the International Committee on Taxonomy of Viruses[8].

In this study, we confirmed the positive reaction of abaca samples of ABTD with anti-BBTV antibody as demonstrated by Thomas and Bajet (1993, unpublished). Then, we showed that the putative causal viruses of the abaca samples affected by ABTD could be detected by PCR using BBTV specific primers. Finally, we concluded that BBTV could be detected from ABTD affected abaca. Additionally, the nucleotide sequences of DNA-R, DNA-S, and DNA-M of BBTV amplified from abaca (BBTV abaca isolates) by PCR were determined for the first time. It was also indicated that the replication initiation protein genes, the coat protein genes, and the movement protein genes of BBTV abaca isolates had very high homologies with those of BBTV banana isolates, and the BBTV abaca isolates belonged to the Asian group, but not the South Pacific group. These results revealed that BBTV, in the Philippines, occurs not only in banana but also in abaca, and BBTV could come and go between banana and abaca.

Molecular detection and characterization of CMV isolated from abaca

One abaca sample, aP81 from which CMV and

Table 7 Sequence homologies (%) of the coat protein gene among CMV abaca and other isolates*

Isolate	Accession no.	aP81	Hawaii	Oahu	Ix	C	FC	FNY	As	O	PR50	Y	China	Kin	TRK-7	WL
aP81	AB250962	-	93	92	94	93	93	93	95	93	93	93	92	76	76	76
Hawaii	U31219	96	-	90	92	99	98	99	93	97	97	96	92	75	75	75
Oahu	U31220	94	92	-	92	90	90	91	91	90	90	90	90	75	74	75
Ix	U20219	97	94	92	-	92	92	92	93	92	92	92	91	75	74	75
C	D00462	96	99	92	94	-	98	99	94	97	97	97	92	75	75	75
FC	D10544	97	97	93	95	98	-	99	93	98	97	97	92	76	75	76
FNY	D10538	98	98	93	95	98	99	-	94	98	97	97	92	76	75	76
As	X77855	98	95	93	95	95	96	97	-	93	93	94	94	77	76	77
O	D00385	97	96	93	96	97	98	98	96	-	97	97	92	75	75	75
PR50	M98501	99	97	94	96	97	98	99	98	98	-	98	92	77	76	77
Y	D12499	97	95	92	95	95	97	97	97	96	98	-	92	76	75	76
China	X65017	95	92	90	93	92	93	94	94	93	94	93	-	75	75	76
Kin	Z12818	81	80	77	80	80	81	81	81	82	82	81	78	-	98	99
TRK-7	L15336	79	78	76	78	78	79	79	79	80	80	79	77	97	-	98
WL	D00463	81	79	77	80	80	80	81	81	81	82	81	78	98	96	-

* Percent identities are presented for nucleotide sequences (above the diagonal) and amino acid sequences (below the diagonal).

BBrMV were detected by ELISA, was used for RT-PCR detection of CMV. Under the annealing condition of 50℃, the target DNA fragment of approximately 800 bp was amplified from aP81 with universal primers that amplify entire viral coat protein gene of the members of the genus *Cucumovirus*[7] (Table 1) (data not shown).

The nucleotide sequence amplified from aP81 was determined following cloning and compared with previously reported 15 isolates of CMV. The putative amino acid sequences of the coat protein gene had higher homologies with CMV subgroup I (94–99%) than with CMV subgroup II (79–81%) (Table 7). In phylogenetic analysis based on the putative amino acid sequences of the coat protein gene, CMV-aP81 belonged to CMV subgroup IB, but not CMV subgroup IA (Fig. 2).

Occurrence of CMV on abaca was experientially known in the Philippines[5], however, there was no study based on detection and characterization of CMV. In this study, we detected CMV from an abaca plant by ELISA and RT-PCR, and showed the CMV isolate was classified into CMV subgroup IB according to the molecular characterization for the first time. This study showed that abaca was included as one of the host plants of CMV as well as banana.

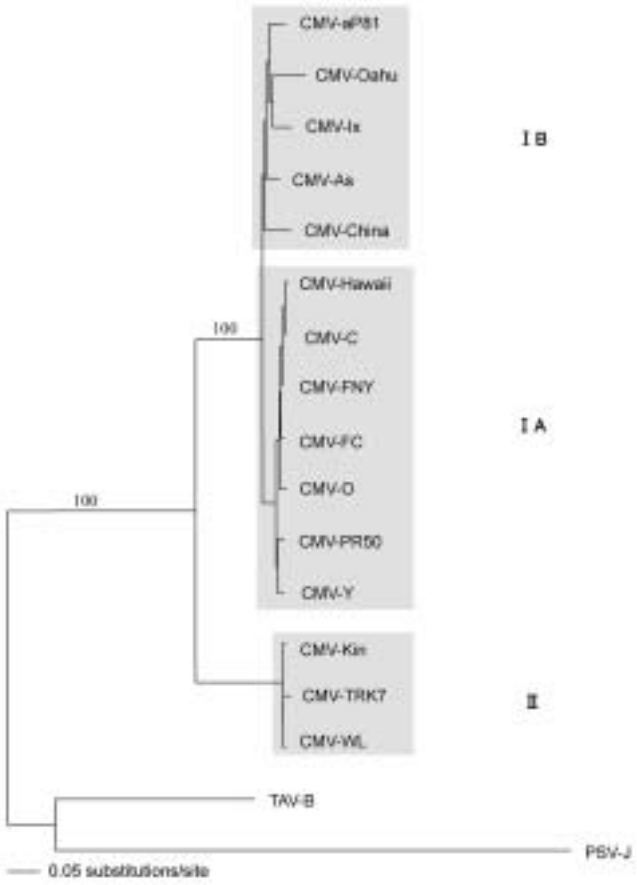

Fig. 2 Additive tree depicting the relationships of *Cucumber mosaic virus* (CMV)-aP81 to other CMVs, *Tomato aspermy virus* (TAV), and *Peanut stunt virus* (PSV) based on the putative amino acid sequences of the coat protein genes.

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アバカ（*Musa textilis* Nee）に発生するバナナバンチ－トップウイルスとキュウリモザイクウイルスの分子生物学的性状の解明

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要約：繊維用作物であるアバカ（*Musa textilis* Nee）は，フィリピンの主要工芸作物の1つである。フィリピンのルソン島とネグロス島のアバカとバナナについて，バナナバンチ－トップウイルス（*Banana bunchy top virus*；BBTV），キュウリモザイクウイルス（*Cucumber mosaic virus*；CMV），および*Banana bract mosaic virus*（BBrMV）の発生状況を調査した。収集したアバカ37株中34株からはBBrMVが，12株からはBBTVが，6株からはCMVが検出された。また，収集したバナナ25株中19株からはBBTVが，14株からはBBrMVが，3株からはCMVが検出された。このことから，本研究で調査した地域のアバカではBBrMVが，バナナではBBTVとBBrMVが主要なウイルスであることが明らかになった。また，バンチ－トップ病のアブラムシ伝搬試験を行い，本病に対して抵抗性を有するアバカの品種を示した。アバカバンチ－トップ病の病原と推定されるウイルスについて，塩基配列を一部決定し，複製開始タンパク質遺伝子，外被タンパク質遺伝子，移行タンパク質遺伝子を既報のウイルスと比較した。アバカから分離されたウイルスは，バナナから分離されたBBTVと各遺伝子のアミノ酸配列で99％以上の高い相同性を示したことから，アバカでもバナナと同様にBBTVが発生していることが，分子生物学的に初めて証明された。また，アバカにおけるCMVの発生をELISA法とRT-PCR法によって初めて確認し，外被タンパク質遺伝子の解析から，CMVサブグループIBに分類されることを示した。

キーワード：アバカ，バナナ，バンチ－トップ病，*Banana bunchy top virus*，フィリピン

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