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Abacá mosaic virus: a distinct strain of *Sugarcane mosaic virus*

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Abstract. Abacá mosaic virus (AbaMV) is related to members of the sugarcane mosaic virus subgroup of the genus *Potyvirus*. The ~2 kb 3′ terminal region of the viral genome was sequenced and, in all areas analysed, found to be most similar to *Sugarcane mosaic virus* (SCMV) and distinct from *Johnsongrass mosaic virus* (JGMV), *Maize dwarf mosaic virus* (MDMV) and *Sorghum mosaic virus* (SrMV). Cladograms of the 3′ terminal region of the NIb protein, the coat protein core and the 3′ untranslated region showed that AbaMV clustered with SCMV, which was a distinct clade and separate from JGMV, MDMV and SrMV. The N-terminal region of the AbaMV coat protein had a unique amino acid repeat motif different from those previously published for other strains of SCMV. The first experimental transmission of AbaMV from abacá (*Musa textilis*) to banana (*Musa sp.*), using the aphid vectors *Rhopalosiphum maidis* and *Aphis gossypii*, is reported. Polyclonal antisera for the detection of AbaMV in western blot assays and ELISA were prepared from recombinant coat protein expressed in *E. coli*. A reverse transcriptase PCR diagnostic assay, with microtitre plate colourimetric detection, was developed to discriminate between AbaMV and *Banana bract mosaic virus,* another *Musa*-infecting potyvirus. Sequence data, host reactions and serological relationships indicate that AbaMV should be considered a distinct strain of SCMV, and the strain designation SCMV-Ab is suggested.

Additional keywords: abacá, banana, RT-PCR diagnosis, ELISA, aphid transmission, potyvirus.

Introduction

The Philippines produces about 75% of the world's abacá or Manila hemp (*Musa textilis*), an important fibre crop grown for both domestic and export purposes (FAOSTAT 2002). A mosaic disease affecting abacá was first described by Calinisan (1934) from the island of Mindanao in the Philippines. It seriously affected abacá production (Eloja and Tinsley 1963) and by 1955, nearly half the area in the Philippines under abacá production was affected, with estimated losses of 25–50% in new plantings common (Stover 1972). The disease remains a serious constraint to abacá production today (Raymundo and Bajet 2000). The disease agent was first identified as a virus (Abacá mosaic virus, AbaMV) by aphid transmission experiments from abacá to abacá and from abacá to *Canna indica* (Ocfemia and Celino 1938).

AbaMV naturally infects *M. textilis* (Musaceae), *C. indica* (Cannaceae) and *Maranta arundinacea* (Marantaceae) (Velasco-Magnaye and Eloja 1966) and experimentally, the host range also includes species in the Poaceae (Celino and Ocfemia 1941; Eloja *et al*. 1962; Velasco-Magnaye and Eloja 1966) and banana (Thomas *et al*. 1997). Aphid vectors include *Aphis gossypii* (Ocfemia and Celino 1938), *Rhopalosiphum nympheae* (Celino 1940) and *Rhopalosiphum (Aphis) maidis* (Celino and Ocfemia 1941) but AbaMV is not transmitted by *Pentalonia nigronervosa* (Celino 1940; Ocfemia *et al*. 1947). AbaMV is not transmitted through true seed of abacá (Calinisan 1939), but is transmitted mechanically and through vegetative propagules such as suckers, corm sections and tissue culture (Diekmann and Putter 1996).

Initially, AbaMV was considered to be a strain of *Sugarcane mosaic virus* (SCMV) (Eloja *et al*. 1962) as it was sap transmissible to maize (Celino and Ocfemia 1941), had morphologically similar particles and was serologically related (Eloja and Tinsley 1963). More recently, the serological relationship with SCMV was confirmed, and other potyviruses including *Maize dwarf mosaic virus* (MDMV), *Sorghum mosaic virus* (SrMV) and *Dasheen mosaic virus* (DsMV) shown to be serologically related (Thomas *et al*. 1997). AbaMV was also shown to be distinct from *Banana bract mosaic virus* (BBrMV), another *Musa*-infecting potyvirus, both serologically and in nucleotide sequence (Thomas *et al*. 1997). The taxonomy

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Table 1. Potyvirus isolates used in PTA-ELISA and RT–PCR assays

Virus	Acronym	DPI ref no.	Host	Origin	Supplier
Johnson grass mosaic virus	JGMV	340[A]	Sorghum	Queensland, Australia	D. M. Persley
Maize dwarf mosaic virus	MDMV	343[A]	Johnson grass	Illinois, USA	R. E. Ford
Sorghum mosaic virus	SrMV	344[A]	Sorghum	Illinois, USA	R. E. Ford
Sugarcane mosaic virus	SCMV	366[A]	Sugarcane	Queensland, Australia	BSES[C]
Banana bract mosaic virus	BBrMV	509[B]	Banana cv. Cardaba	Mindanao, Philippines	Authors
Banana bract mosaic virus	BBrMV	513[B]	Banana cv. Nendran	India	K. Jagadish Chandra
Banana bract mosaic virus	BBrMV	Q1107[B]	Abacá	Luzon, Philippines	E. O. Oloteo, R. V. J. Abgona
Abacá mosaic virus	AbaMV	543[B]	Unknown	Luzon, Philippines	N. Bajet
Abacá mosaic virus	AbaMV	720[B]	Abacá	Mindanao, Philippines	Authors
Abacá mosaic virus	AbaMV	905[B]	Abacá	Mindanao, Philippines	Authors
Abacá mosaic virus	AbaMV	515.3[B]	Abacá	Mindanao, Philippines	Authors
Abacá mosaic virus	AbaMV	730[B]	Abacá	Mindanao,Philippines	Authors
Mixed sample	AbaMV + BBrMV	904[B]	Abacá	Mindanao, Philippines	Authors

[A]Freeze dried tissue.
[B]Fresh or frozen tissue.
[C]Bureau of Sugar Experiment Stations, Brisbane.

of potyviruses infecting the Poaceae has recently undergone revision. SCMV was originally thought to be a single virus comprising a number of host-adapted strains, especially from sugarcane, sorghum and maize. However, Shukla *et al*. (1992) considered these strains to form a subgroup of the genus *Potyvirus* comprising SCMV, MDMV, SrMV and *Johnson grass mosaic virus* (JGMV). This was based on host reactions, cytopathology, amino acid sequencing and peptide profiling of coat proteins, cell-free translations of RNAs and nucleotide sequence analyses. A further novel member of the subgroup, *Zea mosaic virus* (ZeMV), has recently been described (Seifers *et al*. 2000). The known host range of SCMV has expanded with the identification of a new strain (SCMV-AOP) that infects African oil palm, in addition to hosts in the *Poaceae* (Morales *et al*. 2002). The designation of AbaMV as a strain of SCMV is inconclusive using only the previously published serological and host range data.

AbaMV cannot be reliably diagnosed by symptoms alone. In recent infections in *Musa*, both AbaMV and BBrMV can produce similar symptoms of chlorotic areas with rusty-red borders (Thomas and Magnaye 2000). A range of symptoms has been described for abacá mosaic disease, including mosaics and various coloured midrib and petiole streaks (Ocfemia and Celino 1938; Eloja *et al*. 1962; Stover 1972). However, BBrMV, first described by Magnaye and Espino (1990), is now known to also infect abacá (Sharman *et al*. 2000*a*) and some symptoms described for abacá mosaic disease are reminiscent of those caused by BBrMV and *Cucumber mosaic virus* in banana. Thus, symptom descriptions reported in the older literature must be viewed with caution.

This paper investigates the relationship between AbaMV and viruses in the SCMV subgroup using serological techniques and nucleotide and amino acid sequence analyses. It also describes the development of serological and PCR assays for the detection of AbaMV, and its differentiation from BBrMV.

Methods

Virus isolates

The details of potyviruses used in this work are listed in Table 1. Transmission experiments with SCMV (isolate 366) were done in Australia using glasshouse-inoculated plants only. Transmission experiments with AbaMV were conducted in the Philippines, using field samples or glasshouse-inoculated source plants.

Aphid transmission

AbaMV. Aphids (*R. maidis* and *A. gossypii*) were starved for 1–3 h before an acquisition access period (AAP) of 15–30 min on infected tissue and were transferred in groups of approximately 20 aphids per test plant, for an inoculation access period (IAP) of 24–48 h. Virus sources for the experiments were either field isolates or infected plants from previous aphid transmission experiments. Field isolates of AbaMV were indexed for the presence of BBrMV by Southern blot analysis (Thomas *et al*. 1997). Test plants included abacá, and banana cultivars Grand Nain (*Musa* AAA group), Lakatan (*Musa* AAA group), Buhutan (*Musa balbisiana,* BB), Umalag (*Musa* AAA group) and Latundan (*Musa* AAB group).

SCMV. Aphids (*R. maidis* and *Myzus persicae*) were starved for 2–3 h before an AAP of 30–60 s on SCMV-infected sweet corn (*Zea mays*) and an IAP of 12 h on healthy test plants. Five banana plants (cv. Williams, *Musa* AAA group, Cavendish subgroup) and five sweet corn plants were each inoculated with approximately 50 aphids. The same numbers of aphids and plants were used as healthy controls, with omission of the AAP following the starvation period.

Virus indexing

Positive transmission of AbaMV and SCMV to test plants was initially based on symptoms. Transmission of AbaMV was confirmed by Southern blot analysis or microtitre plate colourimetric detection (MTPD) of PCR products using an AbaMV specific probe and/or by plate-trapped antigen ELISA (PTA-ELISA) (Sharman *et al*. 2000*b*; Thomas *et al*. 1997). SCMV transmission was confirmed by PTA-ELISA as described below. Absence of BBrMV was monitored by MTPD of PCR products, using a BBrMV-specific probe (Sharman *et al*. 2000*b*).

Reverse transcriptase (RT)–PCR and microtitre plate detection (MTPD)

All RT–PCR steps were performed in a Hybaid Omnigene thermal cycler (Hybaid Ltd, Teddington, Middlesex, UK). Templates for RT–PCR were partially purified virus preparations (Geering *et al*. 2000), total nucleic acid extracts (Thomas *et al*. 1997) or immuno-captured virions (Sharman *et al*. 2000*b*). For immunocapture, PCR tubes were coated with 50 µL of a 1:1000 dilution of SCMV polyclonal antiserum (strain D, R. Toler). Sap extracts were prepared at the rate of 0.2 g/mL in extraction buffer (0.2 M potassium phosphate, pH 7.0; 10 mM EDTA; 2% PVP; 2% PEG 6000 and 0.4% sodium sulphite). First strand cDNA synthesis was initiated by addition of 10 pmol of oligo d(T) primer (Gibbs and Mackenzie 1997) and water to a final volume of 10 µL then incubation at 80°C for 10 min. Next, 5 × first strand buffer (Invitrogen), 10 mM DTT, 500 µM of each dNTP, 20 U of RNAguard (Amersham) RNase inhibitor, 100 U of Superscript II (Invitrogen) and 3 µg of BSA were added, and reactions incubated at 50°C for 50 min followed by 70°C for 15 min. The PCR mix contained 2.0 µL of cDNA, 12.5 pmol of primer U341 (Langeveld *et al*. 1991), 10 pmol of primer oligo dT, 10 × buffer (Invitrogen), 1.75 mM $MgCl_2$, 200 µM of each dNTP and 1.5 U of Taq polymerase (Invitrogen). Reactions were incubated for 33 cycles of 94°C for 30 s, 56°C for 1 min and 72°C for 1min before a final incubation of 72°C for 3 min.

RT–PCR products from potyvirus isolates (Table 1) and appropriate healthy controls were screened with digoxygenin (DIG)-labelled virus specific probes in a MTPD assay, essentially as described by Sharman *et al*. (2000*b*). An AbaMV-720 specific probe was amplified from the recombinant plasmid 720pp7 by PCR using the primers ABAMVUF1 (5′-TCT TCT GGA AAC CCT GTT T-3′) and ABAMVUR1 (5′-ACC AAG AGA CTC GCA GCA-3′) which were designed to the 3′ untranslated region (UTR). BBrMV specific probes were amplified from isolates 509 and 513 by RT-PCR using U341 and oligo-d(T) primers. Template for BBrMV probe preparation was immuno-captured virions as described previously. A 1:1 mix of probes prepared to BBrMV-509 and -513 was necessary to ensure detection of known BBrMV variants (Sharman *et al*. 2000*b*). The probes were gel purifed as per the manufacturer's instructions (QIAEX II Gel Extraction Kit, Qiagen). After incubation with the probes, the plates were washed under conditions of high stringency as described for the Southern blots, with excess probe removed by three 10 min washes at 65°C in 0.1 × SSC containing 0.1% SDS and a final rinse with PBS-T.

Cloning and sequencing

A fragment from AbaMV isolate 720, approximately 2 kb in size, was amplified by RT–PCR using the potyvirus group specific degenerate primer Pot 2 (Colinet and Kummert 1993) and an oligo d(T) primer. First-strand cDNA synthesis was essentially as described by Thomas *et al*. (1997) and PCR was done using the Elongase enzyme mix (GIBCO BRL, Life Technologies) as per the manufacturer's instructions. Cycling parameters were 94°C for 1 min, 35 cycles of 94°C for 45 s, 56°C for 1 min, 72°C for 2 min and a final extension incubation of 72°C for 3 min. For RT–PCR amplification of isolate 543, the potyvirus group specific degenerate primers U341 and D341 (Langeveld *et al*. 1991) were used to amplify a 341 bp region of the core of the coat protein. Alternatively, primer U341 was used with an oligo d(T) primer to amplify the approximately 700 bp 3′ terminal region of the genome of isolates 515.3 and 904. The RT-PCR protocol was as described by Thomas *et al*. (1997).

PCR products were analysed by electrophoresis in a 1% agarose gel in 0.5 × Tris-borate-EDTA (TBE), followed by staining with ethidium bromide (Sambrook *et al*. 1989). Amplified fragments from AbaMV isolates were cloned using the Original TA Cloning Kit (Invitrogen Corporation, San Diego, CA) as per the manufacturer's instructions. Initial selection of clones was by insert size and presumptive viral inserts were confirmed by cycle sequencing undertaken by the Australian Genome Research Facility, Brisbane. The insert (approximately 2 kb) of recombinant plasmid 720pp5 was digested with *Sac*I (Boehringer Mannheim) and subcloned into a pCRScript SK (+) vector (Stratagene Inc.). The subclones were subsequently sequenced and specific internal primers were designed to complete sequencing of the entire 2 kb insert. Plasmid 720pp7, also approximately 2 kb, was also sequenced using these specific internal primers, and the sequence of this clone lodged as GenBank accession number AY222743. Other partial sequences lodged were for AbaMV isolates 515.3, 543 and 904 (Table 2).

Protein expression

The coat protein coding region of AbaMV-720 was amplified by PCR from the recombinant plasmid 720pp5 and cloned into the expression vector pQE-30 as per the manufacturer's instructions (QIA*expressionist*; Qiagen). The downstream primer was located in the 3′ UTR to allow normal termination of the coat protein. Ligation and cloning of the coat protein gene was as per the manufacturer's instructions and identity of inserts was confirmed via automated sequencing. Batch purification of the expressed protein was performed under native conditions and confirmed by western blot analysis using SCMV polyclonal antiserum (strain D, R. Toler). The protein was further purified and concentrated using Micron 30 spin tubes (Amicon Inc.), with 0.05 M sodium phosphate pH 7.0, as the diluent, and final yield estimated using the BioRad Bradford Assay (BioRad Laboratory Pty Ltd) as per the manufacturer's instructions.

Antibody production

Two polyclonal antisera to the expressed coat protein of AbaMV were prepared using rabbits. For the first (AS-1), three intramuscular injections were administered, each containing ∼300 µg expressed coat protein and ∼33 µg Gerbu adjuvant (Gerbu Biochemicals GmbH, Germany) reconstituted in phosphate buffered saline (PBS). For the second (AS-2), a series of four subcutaneous injections, each of 1 mg expressed coat protein, were given at 3-week intervals. The first injection was emulsified with Freund's complete adjuvant, the remainder with Freund's incomplete adjuvant. The bleeds taken 10 days (AS-1) or 14 days (AS-2) after the third injection, were used. The specificity of antibodies from AS-1 was evaluated by western blot analysis, and for AS-2 by ELISA, as described below.

Serology

Western blot analysis. Leaf samples containing AbaMV-720, SCMV-366 and appropriate healthy controls were partially purified essentially as described by Geering *et al*. (2000). Protein extracts were separated by polyacrylamide gel electrophoresis and transferred to Hybond ECL nitrocellulose membrane using a BioRad Mini Transblot apparatus (BioRad Laboratory) as per the manufacturer's instructions. The membranes were blocked overnight at 4°C, in PBS containing 5% skim milk, then incubated for 1 h at room temperature with detecting polyclonal antibody (diluted 1:1000 in PBS containing 1% skim milk). AbaMV AS-1 or SCMV polyclonal antiserum (strain D, R. Toler) was used for detection. The membranes were washed five times, 5 min each time, in PBS-T and then incubated for 3 h at room temperature with goat anti-rabbit alkaline phosphatase conjugate (GAR-AP) (Sigma Chemical Co.) diluted 1:30 000 in PBS-T containing 1% skim milk. The membranes were washed as before and then developed using the BioRad alkaline phosphatase conjugate substrate kit (BioRad Laboratory) as per the manufacturer's instructions.

Plate-trapped antigen (PTA)-ELISA. AS-2 was used for all ELISA tests, as AS-1 had insufficient titre for use in this assay. Polyclonal

Table 2. Potyvirus isolates included in sequence analyses

Virus[A]	Original host	Strain/isolate[B]	Acroynm[B]	Origin	Accesssion code[C]
AbaMV	Abacá	515.3	AbaMV	Philippines	AY434731
	Abacá	543	AbaMV	Philippines	AY434732
	Abacá	720	AbaMV	Philippines	AY222743
	Abacá	904	AbaMV	Philippines	AY434733
SCMV	Sugarcane	A	SCMV-A	USA	U57354
	Sugarcane	B	SCMV-B	USA	U57355
	Sugarcane	D	SCMV-D	USA	U57356
	Sugarcane	E	SCMV-E	USA	U57357
	Sugarcane	SC	SCMV-SC	Australia	D00948
	Sugarcane	Isis 2	SCMV-I2	Australia	AF006729
	Sugarcane	Nambour 7	SCMV-N7	Australia	AF006733
	Sugarcane	Louisiana	SCMV-USAL	USA — Louisiana	AF006736; U84580
	Sugarcane	Florida	SCMV-USAF	USA — Florida	AF006737; U84579
	Sugarcane	South Africa	SCMV-SA	South Africa	AF006738; U84578
	Sugarcane	LP	SCMV-LP	China	AJ310102
	Sugarcane	YH	SCMV-YH	China	AJ310104
	Maize	MDB	SCMV-MDB	USA	D00949, A34976
	Maize	Boetzingen	SCMV-Bo	Germany	X98168
	Maize	Seehausen 288	SCMV-S288	Germany	X98166
	Maize	Zhejiang	SCMV-Ch	China	AJ271085
	Maize	Leida	SCMV-Sp	Spain	AJ311169
	Maize	Bg	SCMV-Bg		AJ006201
	Maize	G951	SCMV-G951		AJ006199
	Maize	GD	SCMV-GD	China	AJ310105
	Maize	HZ	SCMV-HZ	China	AJ297628
	African oil palm	AOP	SCMV-AOP	Colombia	AY07881
MDMV	Maize	A	MDMV-A		U07216
	Maize	Bg	MDMV-Bg	Bulgaria	AJ001691
SrMV	Sorghum	SCI	SrMV-SCI		U57359
	Sorghum	SCH	SrMV-SCH		U57358
	Sugarcane	YH	SrMV-YH	China	AJ310198
	Sugarcane	XOS	SrMV-XOS	China	AJ310197
JGMV	Johnson grass	JG	JGMV-JG		XO5040
	Johnson grass	VVPS	JGMV-VVPS		Z26920
	Johnson grass	KSI	JGMV-KSI		U07218
ZeMV			ZeMV		AF228693
BBrMV	*Musa* spp.		BBrMV		AF071590
PVY	Potato	Common strain — O	PVY-O		U09509

[A]Virus acronym; AbaMV = Abacá mosaic virus, BBrMV = *Banana bract mosaic virus*, JGMV = *Johnsongrass mosaic virus*, MDMV = *Maize dwarf mosaic virus*, PVY = *Potato virus Y*, SrMV = *Sorghum mosaic virus*, SCMV = *Sugarcane mosaic virus.*
[B]Isolate information provided where no strain designation is stated.
[C]EMBL or GenBank accession code.

antisera to the following potyviruses (supplier in parentheses) were also used in PTA-ELISA (Mowat and Dawson 1987): SCMV (strain D; R. Toler); SrMV (strain SCMV-I; R. Toler); JGMV (strain SCMV-JG; D. Shukla); MDMV (strain A; R. Toler) and BBrMV (Thomas *et al*. 1997). Nunc Maxisorb II microtitre plates were used, with 100 µL reaction volumes, and plates were washed three times, 3 min each wash, with PBS-T between each incubation step.

The plant tissue samples were ground at the rate of 1 g/50 mL in 0.05 M sodium carbonate buffer, pH 9.6, containing 0.1% sodium sulphite and briefly centrifuged before loading into duplicate wells and incubating overnight at 5°C. Antisera were diluted 1:5000 (except AbaMV and BBrMV — 1:2000) in a healthy sap extract (leaf tissue in PBS-T, 1 g/50 mL). Plates were incubated with cross-absorbed polyclonal antiserum and bound polyclonal antibodies were detected with GAR-AP conjugate diluted 1:30 000 in PBS-T. Antibody and conjugate incubations were for 2–3 h at room temperature and substrate development monitored as described previously for MTPD. Reactions are reported as the mean A_{410nm} of at least duplicate wells.

Sequence analyses

Software programs used for sequence analyses were from the Australian National Genomic Information Service (ANGIS), University of Sydney. Multiple sequence alignments were done with CLUSTALW (Thompson *et al*. 1994) and sequence identities calculated using the GCG program DISTANCES. Phylogenetic analyses were done using the PROTPARS program in the PHYLIP (Felsenstein 1985) software package and reliability of cladograms was determined by 100 rounds of bootstrap analysis with input sequences randomised and jumbled three

times. Virus isolates used in the sequence analysis are listed in Table 2 with their GenBank/EMBL accession code.

The nucleotide sequence of the 3′ UTR and amino acid sequences of the coat protein core (equivalent to N_{33}-R_{248} in PVY) (Ward *et al*. 1995) and 3′terminus of the NIb region (equivalent to N_{319}-Q_{501} in PVY) were analysed. Variation between AbaMV isolates was evaluated by comparison of partial coat protein amino acid sequences (equivalent to C_{185}-E_{280} in AbaMV).

Results

Biological properties

Abacá infected with AbaMV showed a range of symptoms. The mosaic pattern of isolate 543 (Fig. 1*a*) was distinct from the chlorotic stripes (Fig. 1*b*) and strong mosaic (Fig. 1*c*) produced by isolate 905. However, isolate 904 was a mixture of BBrMV and AbaMV, and the symptoms it caused were dominated by spindle-shaped chlorotic lesions typical of those reported for BBrMV infection (Fig. 1*d*). Other symptoms observed included chlorotic streaks (isolate 720), necrotic open ovals/streaks with chlorotic centres parallel to minor veins, and rusty necrotic streaks and patches (isolate 515.1).

AbaMV was transmitted from abacá to the banana cultivars Grand Nain, Umalag and Latundan using *A*. *gossypii*, and from abacá to the cultivars Grand Nain, Buhutan and Lakatan by *R*. *maidis*. Symptoms in banana resembled those produced by AbaMV in abacá with isolate 743 (Fig. 1*f*) but were reminiscent of BBrMV for isolate 720 (Fig. 1*e*). The virus was also transmitted from

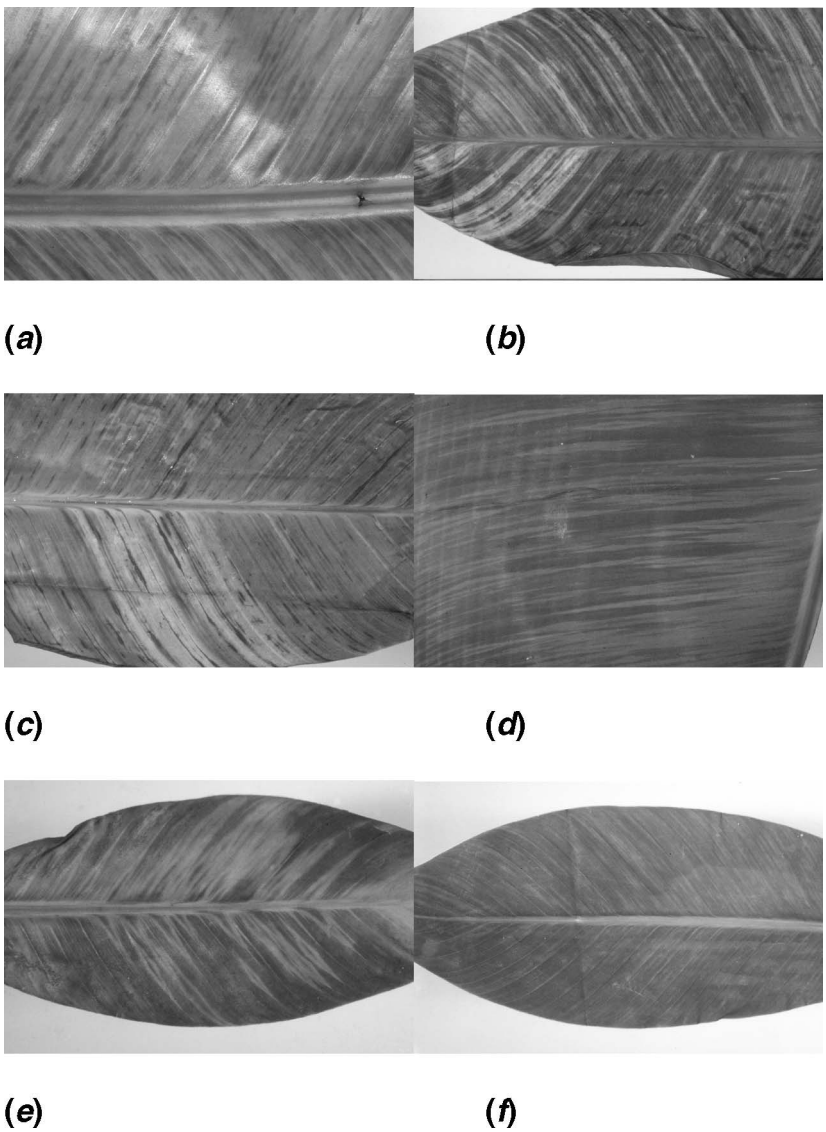

Fig. 1. Symptoms displayed in abacá leaves with samples (*a*) AbaMV-543; (*b, c*) AbaMV-905; (*d*) sample 904, a mixed infection of AbaMV and BBrMV; (*e*) in banana cv. Lakatan (AA) with AbaMV-720; and (*f*) in banana cv. Buhutan (BB) with AbaMV-743.

Table 3. Serological relationships between a range of potyviruses assessed by PTA–ELISA

Virus[A]	Isolate	Host	Antiserum				
			AbaMV	JGMV	SCMV	MDMV	SrMV
AbaMV	720	Abacá	**0.82**[B,C]	0.02	0.28	0.17	0.29
AbaMV	730	Abacá	**1.05**	0.02	0.40	0.27	0.43
JGMV	340	Sorghum	0.03	**1.26**	0.22	0.11	0.38
SCMV	366	Sugarcane	0.18	0.01	**0.67**	0.29	0.12
MDMV	343	Johnson grass	0.03	0.34	0.05	**0.65**	0.44
SrMV	344	Sorghum	0.05	0.23	0.03	0.04	**0.82**

[A]See Table 1 for virus acronyms.
[B]Mean A_{410nm} values, homologous virus/antiserum combinations in bold.
[C]Healthy controls gave A_{410nm} values of 0.01–0.02 in all cases.

the banana cultivars Grand Nain and Lakatan to abacá by *A. gossypii*. SCMV-366 was not transmitted to the banana cultivar Williams using the aphids *R. maidis* or *M. persicae* but was transmitted to sweet corn by both aphid species.

Protein expression

A recombinant protein was produced from the coat protein-coding region of the AbaMV isolate 720 and the purified protein (including the 6 × histidine tag) was similar in size (M_r ~40000, results not shown) to that calculated from the derived amino acid sequence (M_r 35952 with the tag). Typical yields were approximately 100 µg of protein purified from 200 mL broth culture.

Serological and diagnostic assays

AS-2 reacted strongly with its homologous antigen in PTA ELISA (Table 3), and also gave positive reactions with 11 other samples of AbaMV from Luzon and Mindanao in the Philippines (results not shown). The antiserum gave a

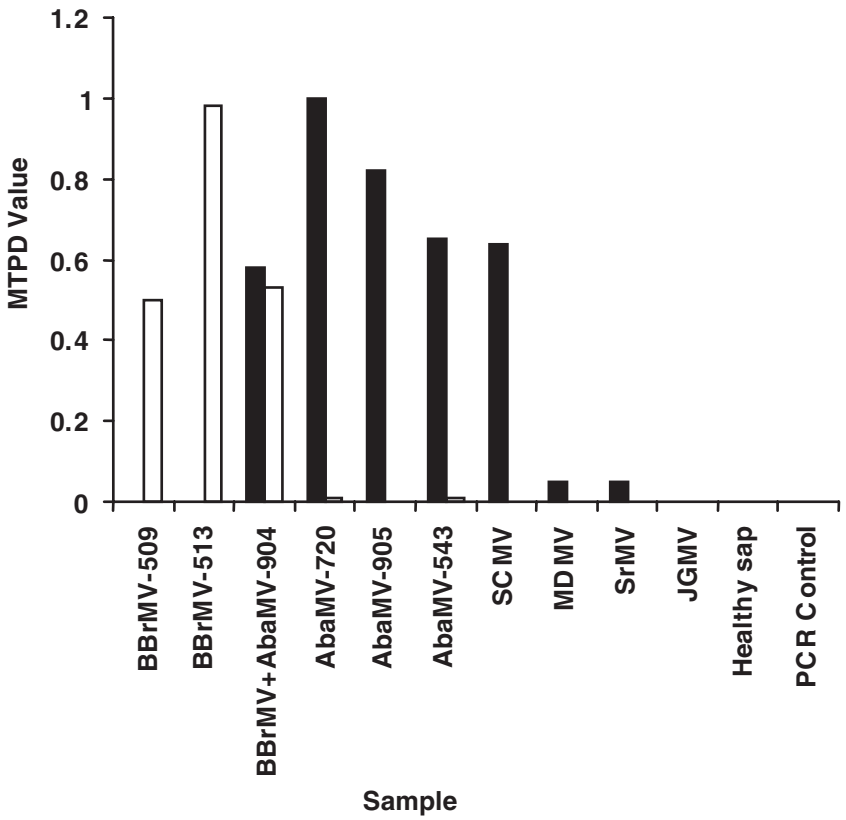

Fig. 2. Detection of potyviruses by immunocapture reverse transcription PCR followed by colourimetric microplate detection using AbaMV probe (closed bars) or BBrMV probe (open bars). Virus acronyms are listed in Table 1.

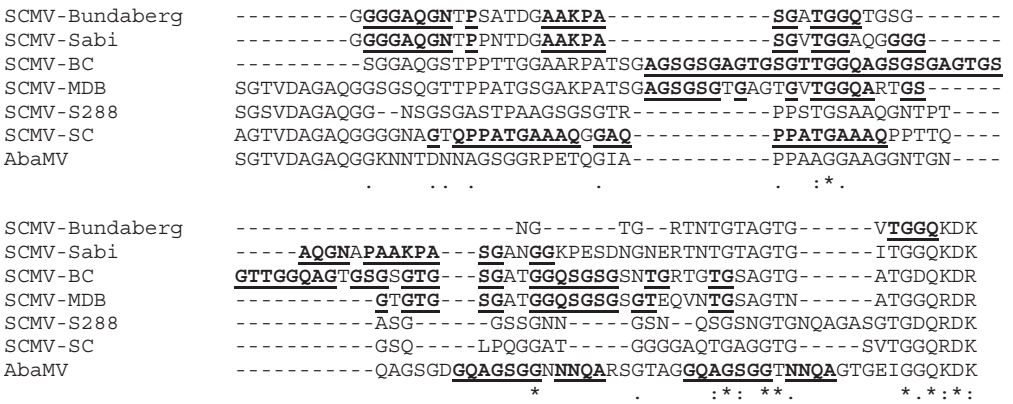

Fig. 3. Amino acid alignment of the N-terminal region of the coat proteins from selected SCMV strains showing repeats and partial repeats in bold and underlined.

relatively weak reaction with SCMV, and negligible reactions with other SCMV subgroup viruses (Table 3) and with BBrMV.

AbaMV did not react with antiserum to JGMV. Comparisons of the homologous and heterologous reactions for the remaining antisera indicated that AbaMV was serologically more closely related to SCMV than to SrMV or MDMV (Table 3). AbaMV was distinct from SCMV, however, as the reaction patterns for these isolates with other antisera did not mirror each other. The strong reaction observed between AbaMV and the SrMV-I antiserum is most likely a result of a high antibody titre and broader cross-reactivity, as reflected by the other heterologous reactions with this antiserum.

In western blot assays, AS-1 detected AbaMV coat protein from partially purified extracts of abacá tissue, whereas no reaction was observed with extracts from healthy abacá, sweet corn or sorghum (results not shown). AS-2 was not used in these assays.

In MTPD, the AbaMV 3′ UTR probe did not react with BBrMV or JGMV but reacted strongly with SCMV, and weakly with MDMV and SrMV (Fig. 2). The percent sequence identities for the 3′ UTR between AbaMV and SCMV were 87–97%, whereas between AbaMV, and MDMV and SrMV they were in the range of 62–67% (Table 4). The BBrMV probe was specific and detected known BBrMV isolates, but did not react with any of the other viruses tested (Fig. 2). A dual AbaMV and BBrMV infection was detected in isolate 904 (Fig. 2) which was confirmed by cloning and sequencing the RT–PCR products.

Sequence analyses

For AbaMV-720, a Q/S cleavage site was identified between the NIb and coat protein regions of the deduced amino acid sequence. Assuming this cleavage point, the coat protein was 328 amino acid residues long and of size M_r 35 237. A DAG aphid transmission motif was located five amino acids downstream of the cleavage site, consistent with other potyviruses. The N-terminal coat protein region of AbaMV had amino acid repeats (Fig. 3) which differed from those published for other SCMV isolates, including SCMV-MDB, SCMV-SC and SCMV-Sabi (Xiao *et al*. 1993; Ward *et al*. 1995). There were no amino acid repeats detected in BBrMV that were similar to those in AbaMV.

The nucleotide sequences from the coat protein core of AbaMV isolates 515.3, 543, 720 and 904 were 97.8–100.0% identical.

In sequence comparisons, AbaMV was most closely related to isolates of SCMV in all areas of the genome examined. The percentage identity values and phylogenetic analyses showed AbaMV was distinct from MDMV, SrMV, ZeMV, JGMV, BBrMV and PVY (Table 4 and Fig. 4). The genetic distance between AbaMV and SCMV isolates from maize or sugarcane varied depending on the part of the

Table 4. Percent identities between AbaMV-720 and a range of potyviruses for amino acid sequence comparisons of the C-terminal region of the NIb protein, the core region of the coat protein (CP) and the nucleotide sequence of the 3′UTR

Virus[A]	NIb[B]	CP core	3′UTR
SCMV[C] — sugarcane	88.6–92.1	92.6–95.4	91.6–94.4
SCMV — maize	91.6–93.6	93.9–97.2	87.5–89.2
SCMV-AOP	–	–	96.9
ZeMV	–	82.4	67.0
MDMV	70.8–71.3	87.0–87.5	61.9–62.8
SrMV	72.3–75.7	85.6–88.4	62.7–66.8
JGMV	52.5	67.6–68.1	–[D]
BBrMV	–	64.2	43.9
PVY	52.7	64.3	41.4

[A] A range of isolates used for SCMV, MDMV, SrMV and JGMV, as detailed in Table 2.
[B] Only the C-terminus region of the NIb (equivalent to N_{319}-Q_{501} in PVY) was analysed.
[C] See Table 1 for virus acronyms.
[D] Sequence was not included in the analyses because it was not comparable in nucleotide length.

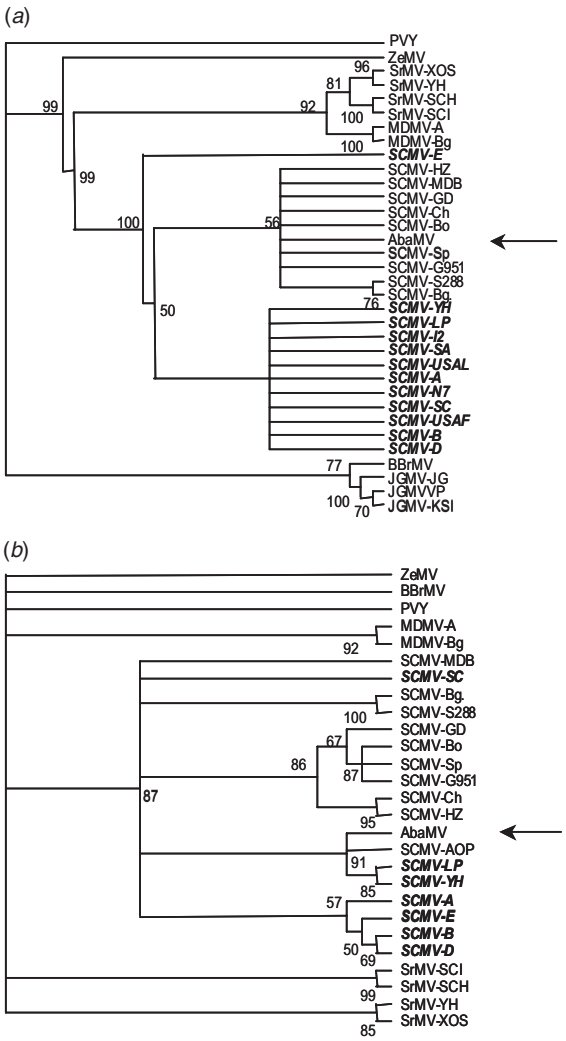

Fig. 4. Cladograms showing the relationship of AbaMV with other potyviruses in (*a*) the core coat protein region (equivalent to N_{33}-R_{248} in PVY); and (*b*) the 3′ UTR. Sugarcane-infecting SCMV isolates are shown in bold and italics and the position of SCMV-Aba indicated with an arrow. Virus acronyms are listed in Table 2. Bootstrap values greater than 50% are indicated and branches with values less than 50% have been collapsed.

genome analysed and AbaMV was not clearly more related to either group. AbaMV was 91.6–93.6% identical to the maize-infecting isolates and 88.6–92.1% identical to the sugarcane-infecting isolates of SCMV, in the C-terminal region of the NIb (Table 4). In the 3′ UTR, the relatedness was reversed and AbaMV was 91.6–94.4% identical to the sugarcane isolates and 87.5–89.2% identical to the maize isolates. In this region, AbaMV was, however, most closely related to the African oil palm-infecting strain, SCMV-AOP (96.9%) and in phylogenetic analyses, AbaMV clustered with SCMV-AOP plus two sugarcane-infecting strains (SCMV-LP and SCMV-YH), separate from the remaining SCMV strains. Identity values between AbaMV and the SCMV isolates in the coat protein core region ranged from 92.6–97.2% (Table 4), and in cladograms, AbaMV clustered with the maize-infecting strains of SCMV, and was separate from the cluster of sugarcane-infecting strains (Fig. 4).

Discussion

AbaMV is clearly a strain of SCMV. It has a typical potyvirus particle morphology, is aphid-transmitted in the non-persistent manner and is serologically related to members of the genus *Potyvirus* (Thomas and Magnaye 2000). Amino acid sequence identity of the core of the coat protein most accurately reflects relationships elsewhere in the genome of potyviruses (Shukla *et al*. 1994). The range of identities between AbaMV and various SCMV isolates for the coat protein core amino acid sequence was 92.6–97.2%. This falls within the proposed range of 90–99% used to define strains of an individual virus (Shukla and Ward 1988), and indicates that AbaMV should be considered a strain of SCMV. The remaining genome regions analysed also revealed strong relationships between AbaMV and the SCMV strains. Levels of identity of 3′ UTR sequences between AbaMV and SCMV isolates was 87.5–96.9%, well above the 80% threshold to define strains proposed by Frenkel *et al*. (1989).

Analysis of the coat protein N-terminal amino acid sequence revealed differences between AbaMV and other strains of SCMV. It has been suggested that host specificity and virulence are determined by the coat protein, especially the N-terminal region, which varies in size and similarity between the SCMV subgroup members (Xiao *et al*. 1993). However, the N-terminal repeat sequences described for the maize-infecting SCMV-MDB (Xiao *et al*. 1993) are not present in maize-infecting isolates of SCMV from Germany (Oertel *et al*. 1997). Also, the repeat sequences present in AbaMV are not present in the only other known *Musa*-infecting potyvirus, BBrMV. AbaMV infects a range of hosts including several *Musa* spp. and maize, but does not contain the repeat motifs present in the other subgroup members infecting these hosts. It appears unlikely that the correlation between repeat sequences and host specificity will be supported after wider comparisons are made.

AbaMV is serologically related to SCMV, SrMV and MDMV, though it was not possible to determine if AbaMV should be considered a strain of SCMV based on these serological reactions alone. The comparison was limited to only one isolate of SCMV and two isolates of AbaMV and thus serological variation between strains was not extensively evaluated. AS-1, prepared with Gerbu adjuvant, was specific for the virus, as demonstrated in western blots. However, the low titre of the antiserum rendered it unsuitable for routine assays such as ELISA (data not shown). By contrast, AS-2, prepared with Freund's adjuvant, was high-titred and suitable for use in ELISA.

Natural hosts of AbaMV include species in the *Musaceae, Cannaceae* and *Marantaceae*, in contrast to most other

strains of SCMV, which are restricted to members of the Poaceae (Teakle *et al*. 1989). On the basis of host range, unique N-terminal repeats in the coat protein and serological relationships, we consider that AbaMV should be considered a distinct strain of SCMV. A suggested strain designation is SCMV-Ab.

The similarity in symptoms caused by AbaMV and BBrMV, and the possibility of mixed infections with the two viruses, combine to make symptoms an unreliable means of identification. AbaMV and BBrMV have now each been shown to infect both banana and abacá. AS-2 reacted strongly with AbaMV and only weakly with SCMV. It gave negligible reactions with other SCMV subgroup viruses and BBrMV, and would thus be a valuable diagnostic reagent for use with abacá and banana viruses. The RT-PCR-MTPD assay developed will also be useful for routine virus indexing as it was able to clearly differentiate AbaMV and BBrMV infections and to detect dual infections of these viruses. The AbaMV-specific probe reacted weakly with the related potyviruses MDMV and SrMV. However, these viruses are not known to infect *Musa* spp. and in the present paper the more closely related SCMV sugarcane isolate was also not transmitted to banana. This cross-reactivity should, therefore, not affect the utility of the assay in practical terms for indexing for AbaMV in *Musa.*

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