Journal of General Virology (2006), **87**, 997–1003 DOI 10.1099/vir.0.81631-0

Corchorus yellow vein virus, a New World geminivirus from the Old World

Cuong Ha,[1] Steven Coombs,[2] Peter Revill,[1]† Rob Harding,[1] Man Vu[3] and James Dale[1]

Correspondence
James Dale
j.dale@qut.edu.au

[1]Tropical Crops and Biocommodities, Institute of Health and Biomedical Innovation, Queensland University of Technology, GPO Box 2434, Brisbane, QLD 4001, Australia

[2]Centre for Information Technology Innovation, Faculty of Information Technology, Queensland University of Technology, Brisbane, QLD 4001, Australia

[3]Department of Plant Pathology, Hanoi Agriculture University, Gia Lam, Hanoi, Vietnam

Received 25 October 2005
Accepted 28 November 2005

A bipartite begomovirus infecting Jute mallow (*Corchorus capsularis*, Tilliaceae) in Vietnam was identified using novel degenerate PCR primers. Analysis of this virus, which was named *Corchorus* yellow vein virus (CoYVV), showed that it was more similar to New World begomoviruses than to viruses from the Old World. This was based on the absence of an AV2 open reading frame, the presence of an N-terminal PWRLMAGT motif in the coat protein and phylogenetic analysis of the DNA A and DNA B nucleotide and deduced amino acid sequences. Evidence is provided that CoYVV is probably indigenous to the region and may be the remnant of a previous population of New World begomoviruses in the Old World.

INTRODUCTION

The *Geminiviridae* are a family of plant viruses with circular single-stranded DNA (ssDNA) genomes encapsidated in twinned particles. Based on their genome arrangement and biological properties, geminiviruses are classified into one of four genera, *Mastrevirus*, *Curtovirus*, *Topocuvirus* and *Begomovirus* (Stanley *et al.*, 2005). Members of the genus *Begomovirus* are transmitted by whiteflies to a wide range of dicotyledonous plants and many have bipartite genomes, known as DNA A and DNA B. DNA A has either one or two open reading frames (ORFs) in the virion sense (AV1, AV2) and up to four major ORFs in the complementary sense (AC1, AC2, AC3, AC4). The DNA B component has one major ORF in each of the virion (BV1) and complementary (BC1) orientations. The DNA A and DNA B components share little sequence similarity, except for ~170 nt of sequence in the intergenic region (IR), termed the common region (CR) (reviewed by Hanley-Bowdoin *et al.*, 1999). Although the CR sequence is usually almost identical in both components, there are examples where the CRs differ substantially between DNA A and DNA B. For example, the CRs of Tomato leaf curl Gujarat virus (ToLCGV) and *Cotton leaf crumple virus* (CLCrV) differed by 40 and 37 %, respectively (Chakraborty *et al.*, 2003; Idris & Brown, 2004). Despite these differences, sequences critical for replication are identical between components of each individual virus. These comprise iterative sequences (iterons) that are recognized and bound by Rep protein (Fontes *et al.*, 1994; Orozco *et al.*, 1998) and a conserved inverted repeat sequence with the potential to form a stem–loop where rolling circle replication initiates (Laufs *et al.*, 1995; Stanley, 1995). Microprojectile bombardment of seedlings with infectious clones of the respective CLCrV and ToLCGV DNA A and DNA B molecules resulted in typical disease symptoms and confirmed that both components are from the same infectious unit (Chakraborty *et al.*, 2003; Idris & Brown, 2004).

Phylogenetic studies show that begomoviruses can be broadly divided into two groups, the Old World viruses (eastern hemisphere, Europe, Africa, Asia) and the New World viruses (western hemisphere, the Americas) (Padidam *et al.*, 1999; Paximadis *et al.*, 1999; Rybicki, 1994). Begomovirus genomes have a number of characteristics that distinguish Old World and New World viruses. All New World begomoviruses are bipartite, whereas both bipartite and monopartite begomoviruses are present in the Old World. In addition, all Old World begomoviruses have an extra AV2 ORF in DNA A that is not present in New World begomoviruses (Rybicki, 1994; Stanley *et al.*, 2005). New World begomoviruses also have an N-terminal PWRsMaGT motif in the coat protein (CP) encoded by AV1, which is absent from Old World begomoviruses (Harrison *et al.*, 2002). In most Old World begomoviruses, there are two iterons upstream of the AC1 TATA box, with a complementary iteron downstream. This downstream iteron is lacking in most New World begomoviruses (Arguello-Astorga *et al.*, 1994).

†Present address: Victorian Infectious Diseases Reference Laboratory, 10 Wreckyn Street, North Melbourne, VIC 3051, Australia.

The GenBank/EMBL/DDBJ accession numbers for the sequences reported in this paper are AY727903 and AY27904.

Rybicki (1994) proposed that most New World viruses arose more recently than Old World viruses and suggested that they may have evolved after the continental separation of the Americas from Gondwana approximately 130 million years ago. Rybicki (1994) speculated that whiteflies moving from Asia to the Americas may have transmitted viruses that were the ancestors of New World viruses that we observe today. These viruses subsequently evolved separately from Old World viruses and this evolution would also have been accompanied by the early loss of the AV2 gene (originally named AV1), which would explain its absence from all New World viruses characterized to date. In more recent times, there is evidence of New World begomoviruses in the Old World and vice versa, due to the increased range of the B biotype of the *Bemisia tabaci* whitefly vector and/or the distribution of infected propagating material. For example, strains of Tomato yellow leaf curl virus (TYLCV) have been identified in the New World (Caribbean Islands and Florida) (reviewed by Czosnek & Laterrot, 1997; Polston *et al.*, 1999) and the New World virus *Abutilon mosaic virus* (AbMV) has been identified in ornamental *Abutilon* spp. in the UK (Brown *et al.*, 2001) and New Zealand (Lyttle & Guy, 2004). However, these are apparently recent introductions and there are no known examples of indigenous viruses from the Old World with genome organization and/or phylogenetic similarity to New World viruses and vice versa. In this paper, we describe the first example of an indigenous Old World begomovirus that has all of the distinguishing characteristics of a New World virus and discuss the ramifications of this finding for current theories on begomovirus evolution.

METHODS

Degenerate primers and PCR. Although degenerate PCR primers have been used to amplify DNA A from a number of begomoviruses, most primer pairs only amplify small fragments of approximately 500 nt in the AV1 gene (Revill *et al.*, 2003; Wyatt & Brown, 1996). To design degenerate primers that would amplify a larger region of DNA A, we aligned begomovirus DNA A sequences from the GenBank database using the CLUSTAL_X program (Thompson *et al.*, 1997) and identified two conserved regions, one at the 5′ end of the AV1 gene (CP) and the other at the 3′ end of the AC1 gene (Rep), approximately 1200 nt apart. Degenerate primers, BegoAFor1 (5′-TGYGARGGiCCiTGYAARGTYCARTC-3′) (i = inosine) and BegoARev1 (5′-ATHCCMDCHATCKTBCTiTGCAATCC-3′), were designed in each region and used in PCRs comprising a 1 μl aliquot of template DNA, 15 mM $MgCl_2$ buffer (Roche), 10 pmol dNTPs, 40 pmol of each primer and 2·5 U *Taq* polymerase (Roche). The reactions were denatured at 94 °C for 5 min and then subjected to 40 cycles at 94 °C (30 s), 50 °C (30 s) and 72 °C (90 s), terminating with 10 min at 72 °C.

The primers were initially tested on total DNA extracted (DNeasy; Qiagen) from several known begomovirus-infected samples from Vietnam, namely *Squash leaf curl virus-China* (SLCCNV), Luffa yellow mosaic virus (LYMV) and TYLCV and in each case a fragment of the expected size (~1·2 kbp) was amplified. Sequence analysis of the cloned amplicon from the SLCCNV-infected sample confirmed the presence of SLCCNV. DNA was subsequently extracted from various samples that had been collected during a virus survey of Vietnam during 2000. These samples included weeds that were exhibiting typical geminivirus symptoms (stunting, bright yellow mosaics and vein yellowing) and Jute (*Corchorus capsularis*), a leaf vegetable and medicinal herb, collected from Hoa Binh province in northern Vietnam, which was showing vein yellowing.

The DNA A-specific primers BegoAFor1 and BegoARev1 amplified a 1·2 kbp product from several of the samples tested, including the Jute sample, which was chosen for further analysis. To amplify DNA B from the Jute sample, the degenerate primer PBL1v2040 (Rojas *et al.*, 1993) was used in combination with an antisense primer (201CRRev 5′-CAGAGACTTTGGTGTGTACC-3′) located in the DNA A IR to amplify a product of ~700 bp. This primer pair was used in a PCR as described above, but at an annealing temperature of 46 °C.

Amplification and cloning of DNA A and DNA B. To amplify the remaining sequence of DNA A and DNA B from the virus infecting Jute, outwardly extending specific primers (DNA A: 201For 5′-TCCTCTTCGAAGAACTCCT-3′, 201Rev 5′-TGTATGAGCAATATCGTGAC-3′; DNA B: 201BFor 5′-GAAGGTATGATGTCTTCCTG-3′, 201BRev 5′-AATCACAATTAGCTCAAGC-3′) were used in PCRs comprising a 1 μl aliquot of template DNA, 15 mM $MgCl_2$ buffer, 10 pmol dNTPs, 40 pmol of each primer and 2·5 U *Taq* polymerase. The reactions were denatured at 94 °C for 5 min, followed by 40 cycles at 94 °C (30 s), 52 °C (30 s) and 72 °C (90 s), terminating with 10 min at 72 °C. For DNA B, the annealing temperature was reduced to 46 °C. The complete DNA A sequence was also amplified using Expand polymerase (Roche) with adjacent outwardly extending primers (201For and 201Rev1 5′-AAAGAACAAAGCAATCAATGAC-3′) at an annealing temperature of 50 °C.

PCR products were gel-purified, ligated into plasmid vector pGEM-T Easy (Promega), introduced into *Escherichia coli* and sequenced. Consensus sequences were determined using the SeqMan program (DNASTAR) and nucleotide and deduced amino acid sequences from three clones for each molecule were analysed using EditSeq (DNASTAR) and Vector NTI. Sequences were compared with the GenBank database using the BLAST programs available at the National Centre for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov/blast). The complete DNA A and DNA B nucleotide sequences and the nucleotide and deduced amino acid sequences of the AC1, AV1, BC1 and BV1 genes were aligned using CLUSTAL_X (Thompson *et al.*, 1997) with analogous sequences from 29 Old World and 11 New World begomoviruses (Table 1). Neighbour-joining trees were generated using TREEVIEW (Page, 1996). Nucleotide identities were calculated with the MegAlign program (DNASTAR) using the CLUSTAL W algorithm.

Replication studies. To confirm that the DNA A and DNA B molecules identified in this study were from the same bipartite begomovirus, replication studies were performed on cloned components. To the best of our knowledge, *Corchorus* yellow vein virus (CoYVV) is not present in Australia and therefore Australian quarantine regulations did not permit co-inoculation experiments with DNA A and DNA B infectious clones. To determine whether the DNA A Rep sequence could initiate replication of DNA B, *Nicotiana tabacum Tnt1 virus* (NT1) cells were co-bombarded with a plasmid expressing the DNA A Rep/TraP/REn sequences encoded by AC1, AC2 and AC3, respectively, and a plasmid containing a 1·5-mer copy of the DNA B molecule.

Constructs

DNA B 1·5-mer replicon. The complete DNA B sequence was amplified by PCR using the Expand Long Template PCR system (Roche Diagnostics) using a pair of adjacent outwardly extending primers, CorBSacFor (5′-GAGCTCCTCTCTCTGTACGACGACCA-3′, nt 448–473) and CorBSacRev (5′-GAGCTCCATGTCTATACCGCATAGTATAC-3′, nt 453–425). PCRs were set up as described above using an annealing temperature of 55 °C and the amplicon was gel-purified (Qiax II; Qiagen) and ligated into the pGEM-T Easy vector

Table 1. GenBank accession numbers for the begomoviruses used in the phylogenetic analysis

Acronym	Species	Accession no.	
		DNA A	DNA B
New World			
AbMV	*Abutilon mosaic virus*	X15983	X15984
BDMV	*Bean dwarf mosaic virus*	M88179	M88180
SLCV	*Squash leaf curl virus*	M38183	M38182
DiYMoV	Dicliptera yellow mottle virus	AF139168	AF170101
MaMPRV	Macroptilium mosaic Puerto Rico virus	AF449192	AF449193
RhGMV	Rhynchosia golden mosaic virus	AF239671	–
SiGMCRV	Sida golden mosaic Costa Rica virus	X99550	X99551
SiGMV	*Sida golden mosaic virus*	AF049336	AJ250731
SiMoV	Sida mottle virus	AY090555	–
SMLCV	Squash mild leaf curl virus	AF421552	AF421553
CaLCuV	Cabbage leaf curl virus	U65529	U65530
ToGMoV	*Tomato golden mottle virus*	AF132852	–
ToMoTV	Tomato mottle Taino virus	AF012300	AF012301
Old World			
ACMV	*African cassava mosaic virus*	AF126802	AF126803
AEV	Ageratum enation virus	AJ437618	–
AYVV	*Ageratum yellow vein virus*	X74516	–
CLCuRV	Cotton leaf curl Rajasthan virus	AF363011	–
EACMV	*East African cassava mosaic virus*	AF126806	AF126807
EpYVV	Eupatorium yellow vein virus	AJ438936	–
ICMV	*Indian cassava mosaic virus*	AJ314739	AJ314740
MYMIV	Mungbean yellow mosaic India virus	AF126406	AF142440
LYMV	Luffa yellow mosaic virus	AF509739	AF509740
MYMV	*Mungbean yellow mosaic virus*	D14703	D14704
PaLCuCNV	Papaya leaf curl China virus	AJ558124	–
PepLCBV	Pepper leaf curl Bangladesh virus	AF314531	–
SACMV	*South African cassava mosaic virus*	AF155806	AF155807
SbCLV	Soybean crinkle leaf virus	AB050781	–
SLCCNV	*Squash leaf curl virus-China*	AF509743	AF509742
StaLCuV	Stachytarpheta leaf curl virus	AJ495814	–
TbLCYNV	Tobacco leaf curl Yunnan virus	AJ566744	–
ToLCLV	Tomato leaf curl Laos virus	AF195782	–
ToLCVV	Tomato leaf curl Vietnam virus	AF264063	–
ToLCV	Tomato leaf curl virus	S53251	–
TYLCTHV	*Tomato yellow leaf curl virus-Thailand*	AY514630	AY514633

to produce pCoY/B-1.0. The fragment containing the potential stem–loop sequence in the DNA B CR was excised from pCoY/B-1.0 and ligated into the pGEM-T Easy vector to form pCoY/B-0.5. The complete DNA B sequence was excised from pCoY/B-1.0 and ligated to pCoY/A-0.5 to form pCoY/B-1.5, which contained the complete DNA B sequence flanked by two DNA B stem–loop sequences.

Rep/TraP/REn gene expression. The complete DNA A sequence was amplified using adjacent outwardly extending primers, CorAPstFor (5′-CTGCAGTTCGTGCATCTGTACTTCTTC-3′, nt 2314–2340) and CorAPstRev (5′-CTGCAGATTGTTCGATCTAT-CCAATCC-3′, nt 2319–2293), as described above. The amplicon was ligated into the pGEM-T Easy vector to produce pCoY/A-1.0. The sequence encompassing the complete AC1 ORF through to the end of the REn gene was amplified using the Expand Long Template PCR system from the pCoY/A-1.0 template, with primers 201RepFor (5′-AGGCACCATGGGAAGTCGTTTTG-3′) and 201REnRev (5′-CTGCACGTGAGATACGGATCTAC-3′). The amplicon was ligated into the pTEST expression vector (a gift from Dr B. Dugdale, Queensland University of Technology) containing a 35S promoter and a Nos terminator in a pGEM-T Easy backbone, to form p35SRep/REn.

Microprojectile bombardment and Southern hybridization. NT1 cells were co-bombarded with either pCoY/B-1.5 alone (1 μg) or pCoY/B-1.5 and p35SRep/REn (0·5 μg) together, as described by Dugdale *et al.* (1998) and harvested 3 days post-inoculation. DNA was extracted using the CTAB method of Stewart & Via (1993) and 40 μg DNA was loaded onto each lane of a 1% agarose gel. Southern hybridization was performed using the DIG (Roche) protocol, with a 1157 nt DNA B probe amplified from the pCoY/B-0.5 plasmid using primers CorBEcoFor (5′-GAATTCAACTGTAGAAC-AATCTCTGTTAG-3′, nt 2021–2043) and CorBSacRev.

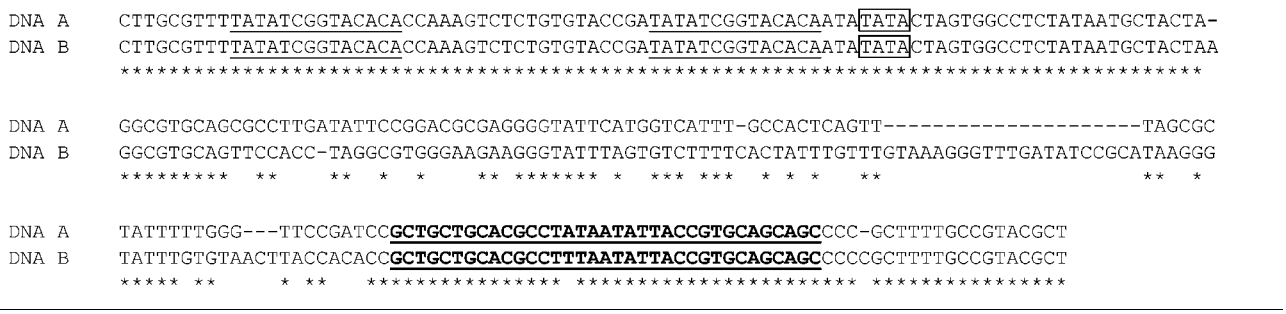

Fig. 1. Comparison of the CR sequences of CoYVV DNA A and DNA B. The putative iteron sequences are underlined, the TATA motif is boxed and stem–loop forming sequences are underlined and in bold. Asterisks indicate identical nucleotides. A comparison of the N-terminal amino acid sequences of the CP of CoYVV and several representative New World and Old World begomoviruses is given in Table 2.

RESULTS

CoYVV sequence

Complete nucleotide sequences of DNA A and DNA B were obtained and we named the virus *Corchorus* yellow vein virus (CoYVV). The DNA A molecule was 2724 nt in length, whereas the DNA B molecule comprised 2691 nt. DNA A encoded one major ORF in the sense orientation (AV1) and four in the complementary sense (AC1, AC2, AC3 and AC4). DNA A did not encode an AV2 ORF. DNA B encoded two major ORFs, BV1 on the virion strand and BC1 on the complementary strand. The CRs of DNA A and DNA B comprised 228 and 254 nt, respectively, with 70·2 % identity. This low identity was due, in part, to a 21 base insertion in the DNA B CR between the TATA box and the stem–loop sequence; the remainder of the CR sequences were 84 % identical. Each CR contained two identical iterons, both upstream of the AC1 TATA box, as well as identical stem–loop sequences that included the conserved TAATATTAC nonanucleotide sequence present in the CRs of all characterized geminiviruses (Fig. 1). A PWRLMAGT motif was identified at the N terminus of the deduced CoYVV CP sequence encoded by AV1 (Table 2).

Replication analysis

Southern hybridization experiments using a DIG-labelled DNA B-specific probe showed that microprojectile bombardment of NT1 cells with a construct expressing the DNA A Rep/TraP/REn sequences initiated replication of DNA B, released from a plasmid harbouring a 1·5-mer copy of DNA B. No DNA B replication was observed in the absence of the Rep/TraP/REn gene product (Fig. 2).

Phylogenetic analysis

BLAST searches and nucleotide sequence alignments showed that CoYVV DNA A was more closely related to New World begomoviruses than to those from the Old World, and with closest overall nucleotide identity (60·2 %) to Macroptilium mosaic Puerto Rico virus (data not shown). Sequence alignments showed that CoYVV DNA B was also more closely related to New World begomoviruses with closest overall nucleotide identity to Tomato mottle Taino virus (ToMoTV; 45·9 %). Higher similarity was observed for the deduced amino acid sequence of the BC1 gene, which was 75 % similar to the analogous sequence of *Bean dwarf mosaic virus* (BDMV) from Columbia (data not shown). In addition, the CoYVV DNA A lacked the AV2 ORF that is present in Old World begomoviruses, but absent from all New World begomoviruses. Other similarities to many begomoviruses from the New World included the presence of a PWRLMAGT motif at the CoYVV CP N terminus and

Table 2. Comparison of the N-terminal amino acid sequences of the CP of CoYVV and several representative New World (the Americas) and Old World (Asia, Africa) begomoviruses (Harrison *et al.*, 2002).

The conserved motif PWRsMaGT is highlighted in bold. The initial methionine residue (M) is the first amino acid of the CP. GenBank accession numbers for these sequences and the virus names are provided in Table 1.

Virus	N terminus of the CP	Origin
MaMPRV	MPKRDA**PWRSSAGT**SKVSRN	America
SiGMV	MPKREL**PWRSMAGT**SKVSRN	America
ToGMoV	MPKRDA**PWRLMGGT**SKVSRS	America
RhGMV	MPKRDA**PWRLSAGT**SKVSRS	America
BDMV	MPKRDA**PWRSMAGT**TKVSRN	America
CoYVV	MPKRDA**PWRLMAGT**SKVSRS	This study
LYMV	MSKRPADIIISTPASKVRRR	Asia
SLCCNV	MSKRPADIIISTPASKVRRR	Asia
ToLCVV	MSKRPADIVISTPASKVRRR	Asia
ICMV	MSKRPADIIISTPASKVRRR	Asia
TYLCTHV	MSKRPADILISTPVSKVRRR	Asia
ToLCLV	MSKRPGDIIISTPVSKVRRR	Asia
ACMV	MSKRPGDIIISTPGSKVRRR	Africa
EACMV	MSKRPGDIIISAPVSKVRRR	Africa

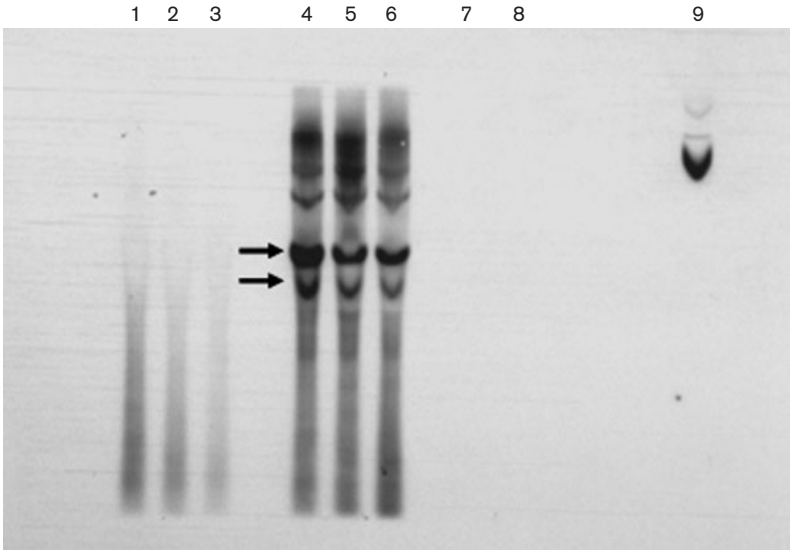

Fig. 2. Southern blot analysis of DNA extracted from NT1 cells bombarded with a 1·5-mer copy of the CoYVV DNA B sequence (pCoY/B-1.5) alone (lanes 1–3), pCoY/B-1.5 co-bombarded with a plasmid expressing the CoYVV Rep/TraP/REn genes (p35SRep/REn) (lanes 4–6), unshot and p35SRep/REn controls (lanes 7 and 8, respectively) and 270 pg pCoY/B-1.5 DNA (lane 9). The blots were hybridized with a DNA B-specific probe. Open circular and supercoiled DNA are indicated by the top and bottom arrows, respectively.

the absence of a complementary iteron downstream of the AC1 TATA box. Phylogenetic analysis using the complete DNA A and DNA B nucleotide sequences showed that CoYVV grouped more closely with New World begomoviruses, but was the most distant of the New World begomoviruses (100 % bootstrap support) (Fig. 3). A similar tree topology was obtained using the AV1 nucleotide and deduced amino acid sequences and the AC1, BC1 and BV1 nucleotide sequences (data not shown).

DISCUSSION

We have identified a bipartite virus from the Old World that is more similar to New World geminiviruses than to other indigenous Old World viruses. This conclusion is based on the absence of an AV2 ORF, the presence of an N-terminal PWRLMAGT motif in the CP, the absence of a complementary iteron downstream of the stem–loop sequence and phylogenetic analysis of the DNA A and DNA B nucleotide

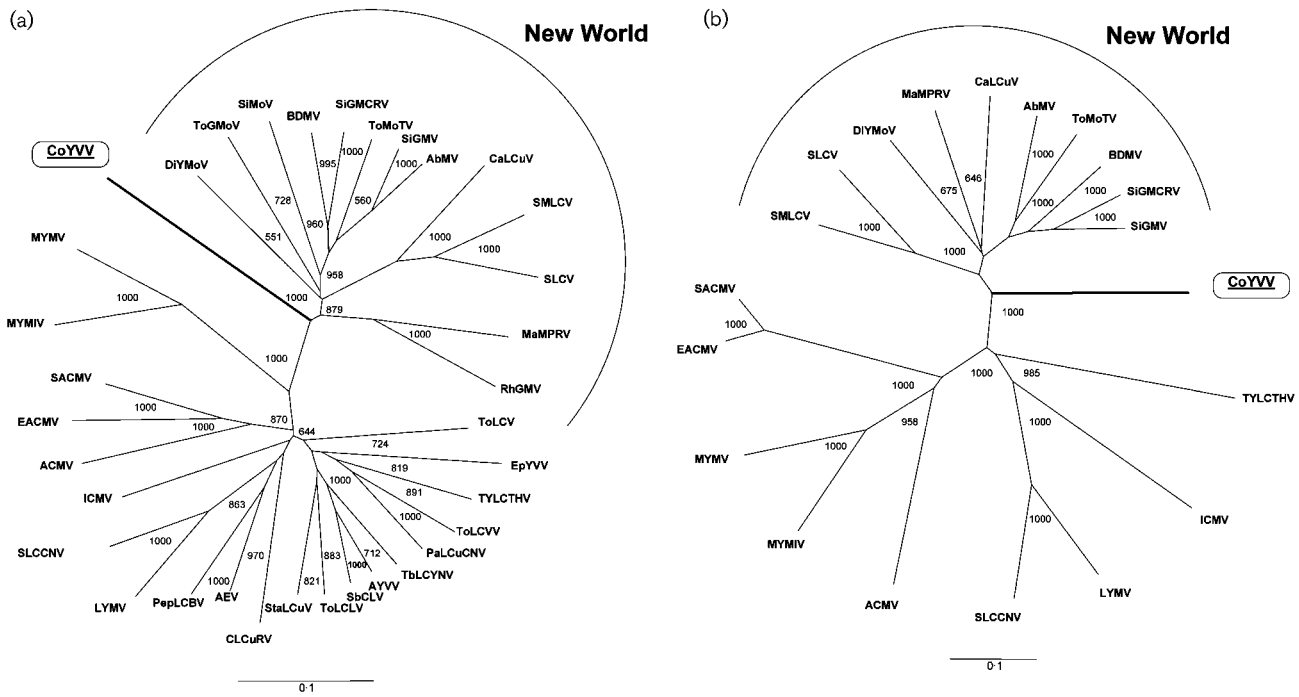

Fig. 3. Phylogenetic analysis of the complete CoYVV DNA A (a) and DNA B (b) nucleotide sequences. CoYVV is circled and underlined. Bootstrap values are indicated (1000 replicates). The full name and GenBank accession numbers for the sequences used in the analysis are presented in Table 1.

and deduced amino acid sequences. Although the nucleotide sequences of the CoYVV DNA A and DNA B CRs were only 70·2 % identical, due in part to a 21 nt insertion in the DNA B CR, they shared identical iterons and stem–loop sequences, suggesting that they represented two components of the one virus. This was supported by microprojectile bombardment of NT1 cells, which showed that a construct harbouring the DNA A Rep/TraP/REn sequence initiated episomal replication of DNA B released from a plasmid harbouring a 1·5-mer copy of the DNA B molecule. Our results confirmed that the CoYVV DNA A and DNA B molecules represented a biologically functional unit from the same begomovirus.

CoYVV is not the only Old World geminivirus to bear some relationship to New World geminiviruses. Phylogenetic analysis of the CP, BC1, BC2 and IRA/IRB sequences of the Old World *Mungbean yellow mosaic virus* (MYMV) showed that they were closely related to viruses from the New World (Rybicki, 1994). Our phylogenetic analysis of the complete DNA A sequence from a large number of Old and New World geminiviruses showed that, whereas MYMV was distal to other Old World viruses, it was still more closely related to Old World geminiviruses than to New World viruses. The complete MYMV DNA B sequence was even more closely related to Old World viruses, whereas the CoYVV DNA A and DNA B sequences were both more closely related to New World viruses. It should also be noted that MYMV encodes an AV2 ORF, although the sequence in GenBank (e.g. accession no. D14703) appears to contain a frameshift error in AV2 that results in two AV2 genes.

The distal position of CoYVV on phylogenetic trees relative to the New World begomoviruses with which it shares closest similarity suggests that CoYVV is not a New World virus that has been recently introduced into Vietnam. Rather, it is more likely that it has been in Vietnam for a considerable period. Jute is a native of southern China (http://www.hear.org/gcw/html/autogend/species/5199.htm) and is propagated as a vegetable and fibre crop by seed, not cuttings. There are no reports of seed transmission of begomoviruses, which suggests that CoYVV has either been transmitted to Jute in Vietnam or CoYVV-infected plants entered Vietnam from nearby southern China. Although some Old World and New World begomoviruses have been detected in the New and Old Worlds, respectively, these are probably recent introductions either as a result of spread of the B biotype of the *B. tabaci* whitefly vector (reviewed by Czosnek & Laterrot, 1997; Polston *et al.*, 1999) or the direct importation of infected plants (Brown *et al.*, 2001; Lyttle & Guy, 2004). Therefore, CoYVV appears to be the first indigenous begomovirus identified from the Old World with closer similarity to New World begomoviruses. Rybicki (1994) suggested that New World viruses may have evolved from Old World viruses after continental separation from Gondwana, possibly as a result of whitefly transmission of ancestral Old World viruses to the New World. Rybicki (1994) also suggested that the absence of the AV2 ORF from all New World bipartite geminiviruses could be explained by its early loss after arrival in the New World and the subsequent evolution of AV2-deficient New World viruses. The occurrence of CoYVV in Vietnam strongly suggests that New World and Old World viruses have been present together in this region for some considerable time. It also suggests that the common ancestor of New World viruses originated in the Old World and that both the New World and Old World begomoviruses had evolved prior to continental separation. It is possible that CoYVV may be a remnant from the population of New World begomoviruses that previously existed in the Old World. Alternatively, the begomoviruses may have evolved in the Old World, and a progenitor of the current New World begomoviruses moved to the New World by unknown means. Although it is possible that whiteflies transmitted a CoYVV-like virus to the Americas, it is tempting to speculate that Asian ancestors of American Indians (for discussion see http://www.hrw.com/science/si-science/biology/evolution/origin/origin.html) or very early Chinese traders may have moved the virus(es) to the New World.

Vietnam appears to be a major centre for plant virus diversity. In previous studies, we have shown that sequence variability of one genome component of the ssDNA Banana bunchy top virus (BBTV) in Vietnam was almost double that observed elsewhere in the world (Bell *et al.*, 2002). High levels of sequence variability were also observed in the ssRNA potyvirus *Papaya ringspot virus* (PRSV; Bateson *et al.*, 2002). We have also previously identified two begomoviruses infecting Vietnamese cucurbits with CP genes that appear to have a recombinant origin (SLCCNV and LYMV; Revill *et al.*, 2003). The discovery of CoYVV further emphasizes the degree of virus diversity present in Vietnam. We are currently characterizing geminiviruses and associated ssDNA molecules infecting a large range of crops and weeds in Vietnam, to determine whether additional viruses similar to CoYVV are present and provide us with further insights into begomovirus evolution.

ACKNOWLEDGEMENTS

This work was funded by the Australian Centre for International Agricultural Research (ACIAR) and the Australian Research Council. The authors thank Brett Williams for assistance with the construction of plasmids for the *in vitro* replication studies and Jennifer Kleidon for maintenance of the NT1 cell lines.

REFERENCES

Arguello-Astorga, G. R., Guevara-Gonzalez, R. G., Herrera-Estrella, L. R. & Rivera-Bustamante, R. F. (1994). Geminivirus replication origins have a group-specific organization of iterative elements: a model for replication. *Virology* **203**, 90–100.

Bateson, M. F., Lines, R. E., Revill, P., Chaleeprom, W., Ha, C. V., Gibbs, A. J. & Dale, J. L. (2002). On the evolution and molecular epidemiology of the potyvirus *Papaya ringspot virus*. *J Gen Virol* **83**, 2575–2585.

Bell, K. E., Dale, J. L., Ha, C. V., Vu, M. T. & Revill, P. A. (2002). Characterisation of Rep-encoding components associated with banana bunchy top nanovirus in Vietnam. *Arch Virol* **147**, 695–707.

Brown, J. K., Idris, A. M., Torres-Jerez, I., Banks, G. K. & Wyatt, S. D. (2001). The core region of the coat protein gene is highly useful for establishing the provisional identification and classification of begomoviruses. *Arch Virol* **146**, 1581–1598.

Chakraborty, S., Pandey, P. K., Banerjee, M. K., Kalloo, G. & Fauquet, C. M. (2003). *Tomato leaf curl Gujarat virus*, a new *Begomovirus* species causing a severe leaf curl disease of tomato in Varanasi, India. *Phytopathology* **93**, 1485–1495.

Czosnek, H. & Laterrot, H. (1997). A worldwide survey of tomato yellow leaf curl viruses. *Arch Virol* **142**, 1391–1406.

Dugdale, B., Beetham, P. R., Becker, D. K., Harding, R. M. & Dale, J. L. (1998). Promoter activity associated with the intergenic regions of banana bunchy top virus DNA-1 to -6 in transgenic tobacco and banana cells. *J Gen Virol* **79**, 2301–2311.

Fontes, E. P., Gladfelter, H. J., Schaffer, R. L., Petty, I. T. & Hanley-Bowdoin, L. (1994). Geminivirus replication origins have a modular organization. *Plant Cell* **6**, 405–416.

Hanley-Bowdoin, L., Settlage, S. B., Orozco, B. M., Nagar, S. & Robertson, D. (1999). Geminiviruses: models for plant DNA replication, transcription, and cell cycle regulation. *CRC Crit Rev Plant Sci* **18**, 71–106.

Harrison, B. D., Swanson, M. M. & Fargette, D. (2002). Begomovirus coat protein: serology, variation and functions. *Physiol Mol Plant Pathol* **60**, 257–271.

Idris, A. M. & Brown, J. K. (2004). *Cotton leaf crumple virus* is a distinct Western Hemisphere begomovirus species with complex evolutionary relationships indicative of recombination and reassortment. *Phytopathology* **94**, 1068–1074.

Laufs, J., Traut, W., Heyraud, F., Matzeit, V., Rogers, S. G., Schell, J. & Gronenborn, B. (1995). *In vitro* cleavage and joining at the viral origin of replication by the replication initiator protein of tomato yellow leaf curl virus. *Proc Natl Acad Sci U S A* **92**, 3879–3883.

Lyttle, D. J. & Guy, P. L. (2004). First record of Geminiviruses in New Zealand: *Abutilon mosaic virus* and *Honeysuckle yellow vein virus*. *Aust Plant Pathol* **33**, 321–322.

Orozco, B. M., Gladfelter, H. J., Settlage, S. B., Eagle, P. A., Gentry, R. N. & Hanley-Bowdoin, L. (1998). Multiple *cis* elements contribute to geminivirus origin function. *Virology* **242**, 346–356.

Padidam, M., Sawyer, S. & Fauquet, C. M. (1999). Possible emergence of new geminiviruses by frequent recombination. *Virology* **265**, 218–225.

Page, R. D. (1996). TreeView: an application to display phylogenetic trees on personal computers. *Comput Appl Biosci* **12**, 357–358.

Paximadis, M., Idris, A. M., Torres-Jerez, I., Villarreal, A., Rey, M. E. & Brown, J. K. (1999). Characterization of tobacco geminiviruses in the Old and New World. *Arch Virol* **144**, 703–717.

Polston, J. E., McGovern, R. J. & Brown, L. G. (1999). Introduction of tomato yellow leaf curl virus in Florida and implications for the spread of this and other geminiviruses of tomato. *Plant Dis* **83**, 984–988.

Revill, P. A., Ha, C. V., Porchun, S. C., Vu, M. T. & Dale, J. L. (2003). The complete nucleotide sequence of two distinct geminiviruses infecting cucurbits in Vietnam. *Arch Virol* **148**, 1523–1541.

Rojas, M. R., Gilbertson, R. L., Russell, D. R. & Maxwell, D. P. (1993). Use of degenerate primers in the polymerase chain reaction to detect whitefly-transmitted geminiviruses. *Plant Dis* **77**, 340–347.

Rybicki, E. P. (1994). A phylogenetic and evolutionary justification for three genera of *Geminiviridae*. *Arch Virol* **139**, 49–77.

Stanley, J. (1995). Analysis of African cassava mosaic virus recombinants suggests strand nicking occurs within the conserved nonanucleotide motif during the initiation of rolling circle DNA replication. *Virology* **206**, 707–712.

Stanley, J., Bisaro, D. M., Briddon, R. W., Brown, J. K., Fauquet, C. M., Harrison, B. D., Rybicki, E. P. & Stenger, D. C. (2005). *Geminiviridae*. In *Virus Taxonomy. VIIIth Report of the International Committee on Taxonomy of Viruses*, pp. 301–326. Edited by C. M. Fauquet, M. A. Mayo, J. Maniloff, U. Desselberger & L. A. Ball. London: Elsevier/Academic Press.

Stewart, C. N., Jr & Via, L. E. (1993). A rapid CTAB DNA isolation technique useful for RAPD fingerprinting and other PCR applications. *Biotechniques* **14**, 748–750.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* **25**, 4876–4882.

Wyatt, S. D. & Brown, J. K. (1996). Detection of subgroup III geminivirus isolates in leaf extracts by degenerate primers and polymerase chain reaction. *Phytopathology* **86**, 1288–1293.