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Coat Protein Gene Replacement Results in Whitefly Transmission of an Insect

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Petra Höfer,* Ian D. Bedford,† Peter G. Markham,† Holger Jeske,* and Thomas Frischmuth*,[1]

*Biologisches Institut, Lehrstuhl für Molekularbiologie und Virologie der Pflanzen, Universität Stuttgart, Pfaffenwaldring 57, 70550 Stuttgart, Germany; and †Department of Virus Research, John Innes Centre, Colney Lane, Norwich NR4 7UH, United Kingdom

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Geminiviruses are transmitted by whiteflies, leafhoppers, or treehoppers. The whitefly species *Bemisia tabaci* (Gennadius) is the most efficient vector of Subgroup III geminiviruses. An isolate of Abutilon mosaic virus (AbMV), a bipartite geminivirus, was not detectable in the DNA extract from insects by Southern blot analysis, nor was the isolate transmissible by the B-biotype of *B. tabaci,* although the virus DNA was amplified (by PCR) from some insects. In contrast, Sida golden mosaic virus (SiGMV-Co), a closely related geminivirus, was acquired and transmitted by *B. tabaci* to various host plants. The coat protein of AbMV was replaced with that of SiGMV-Co. The resulting chimeric AbMV was acquired and transmitted to various host plants by *B. tabaci*, indicating that the coat protein plays an essential role in the transmission process by *B. tabaci*.
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INTRODUCTION

Geminiviruses are small plant viruses with circular single-stranded DNA genomes encapsidated in twinned (geminate) particles (Harrison, 1985). Members have been divided into three genera (Subgroups I, II, and III) (Briddon and Markham, 1995). Subgroup III members infect dicotyledonous plants, are whitefly-transmitted, and normally have bipartite genomes (DNAs A and B). DNA A encodes the coat protein (AV1) as well as proteins required for replication (AC1), transcription transactivation (AC2), and replication enhancement (AC3) (Lazarowitz, 1992; Timmermans *et al.,* 1994). DNA B enhances disease symptoms but plays no role in DNA replication. The two gene products (BV1 and BC1) encoded by this component are involved in virus spread through nuclear shuttling and cell to cell movement, symptom production, and host range (von Arnim and Stanley, 1992; Ingham and Lazarowitz, 1993; Noueiry *et al.*, 1994; Ingham *et al.*, 1995; Schaffer *et al.,* 1995).

For geminiviruses, the interaction between virus and vector is described as circulative (Harrison, 1985) and nonpropagative (Boulton and Markham, 1986). The virus has to pass through specific cells within the gut to enter the hemolymph before passing out of the insect during feeding, via the salivary glands (Markham *et al.*, 1994). The transmission cycle can therefore be considered in three phases: ingestion, acquisition, and inoculation. No evidence has been obtained that geminiviruses have any specific proteins analogous to the aphid-transmission assistor proteins of potyviruses or caulimoviruses, but geminiviruses may be transmitted like luteoviruses, which depend on the coat protein interacting with receptors in the salivary glands (Gildow, 1987). Luteoviruses require capsid integrity with an extended structural (readthrough) protein within the capsid to determine vector specificity (Wang *et al.*, 1995). In one instance, movement protein(s) of a bipartite geminivirus has been implicated as having an indirect role in the acquisition process (Liu *et al.*, 1997) but it has been shown that the major role is played by the coat protein in the form of the virion. Within the whitefly family the most important vectors are in the genus *Bemisia* and the species *B. tabaci* is able to transmit most Subgroup III geminiviruses (Harrison, 1985; Markham *et al.*, 1994; Bedford *et al.*, 1994). However, some geminiviruses from ornamental plants, such as Abutilon mosaic virus (AbMV) and honeysuckle yellow vein virus (Bedford *et al.*, 1994; Wu *et al.*, 1996), and some clones (Liu *et al.*, 1997) are no longer vector transmissible. Acquisition and retention within *B. tabaci* have been studied for several geminiviruses, often by means of DNA-based techniques (Polston *et al.*, 1990; Zeidan and Czosnek, 1991; Azzam *et al.*, 1994; Metha *et al.*, 1994; Caciagli *et al.*, 1995). Cohen *et al.* (1989) showed that nonvector-whiteflies could acquire virions and others have confirmed this nonspecific acquisition, using DNA-based techniques (Polston *et al.*, 1990; Liu *et al.*, 1997). These techniques also showed that acquisition and retention by whiteflies of some geminiviruses could still occur even if the viruses were not insect-transmissible (Wu *et al.*, 1996).

The coat protein is essential for transmission and in

[1] To whom correspondence and reprint requests should be addressed. Fax: 49-711-685-5096. E-mail: thomas.frischmuth@po.uni-stuttgart.de.

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determining insect specificity. Exchange of the African cassava mosaic virus (ACMV) coat protein gene with that of beet curly top virus (BCTV) altered the insect specificity of ACMV from whiteflies to leafhoppers (Briddon *et al.*, 1990). Whiteflies have been shown to be unable to acquire coat protein mutants of geminiviruses which did not form capsids (Azzam *et al.*, 1994; Liu *et al.*, 1997). In this paper we describe the effect of replacing the coat protein gene of an insect-nontransmissible isolate of AbMV with that of an insect-transmissible isolate of Sida golden mosaic virus from Costa Rica (SiGMV-Co).

MATERIALS AND METHODS

Plants and viruses

The AbMV-infected *Abutilon sellovianum* plants have been described earlier (Jeske and Werz, 1980). SiGMV-Co-infected *Sida rhombifolia* plants were collected from Costa Rica (Bedford *et al.*, 1994).

Construction of infectious clones

Recombinant DNA techniques were performed as described by Sambrook *et al.* (1989). Restriction endonucleases and DNA-modifying enzymes were used as recommended by the manufacturers. The sequence and adjacent sequences of the replaced coat protein of AbMV were determined using either the dideoxynucleotide chain termination method with the Pharmacia sequencing kit and [α-^{35}S]dATP or by automatic sequence analysis with the Li-Cor system according to the manufacturer's instructions.

Infectious clones of AbMV (Frischmuth *et al.*, 1990) and SiGMV-Co (Höfer *et al.*, 1997) have been described. The coat protein gene of AbMV was replaced with that of SiGMV-Co by removal of the coat protein coding region of AbMV by polymerase chain reaction (PCR) amplification and subsequent insertion of the PCR-amplified coat protein gene of SiGMV-Co. An AbMV genome was deprived of the coat protein coding region by amplification with the primer pair AbCP3 (5′-GGT TAA AGC TCG TGG GCC-3′, located at position 353–336) and AbCP4 (5′-ATT TGA ATT TTA TTG AAT GAT TT-3′, located at position 1118–1140) from a full-length *Pst*I clone in pBluescript KS+. The primers are in opposite orientation in the AbMV sequence (Fig. 1; Frischmuth *et al.*, 1990). The PCR reaction mixture of 100 μl consisted of 100 ng template, 10 m*M* Tris–HCl (pH 8.8), 50 m*M* KCl, 200 μM of each dNTP, 2.5 m*M* $MgCl_2$, 50 p*M* of each primer, and 2 units of *Taq* DNA polymerase (Eurobio). Viral DNA was amplified by 30 cycles of 1 min at 95°, 1 min at 55°, and 1.5 min at 72°. The coat protein gene of SiGMV-Co was amplified from a DNA A full-length *Pst*I clone in pBluescript KS+ with primers SGCP1 (5′-TTT ATT AAT TCA TTA TCG-3′) and SGCP2 (5′-CAA AAT GCC TAA GCG CGA-3′), located at position 1092–1075 and 329–346 in the SiGMV-Co

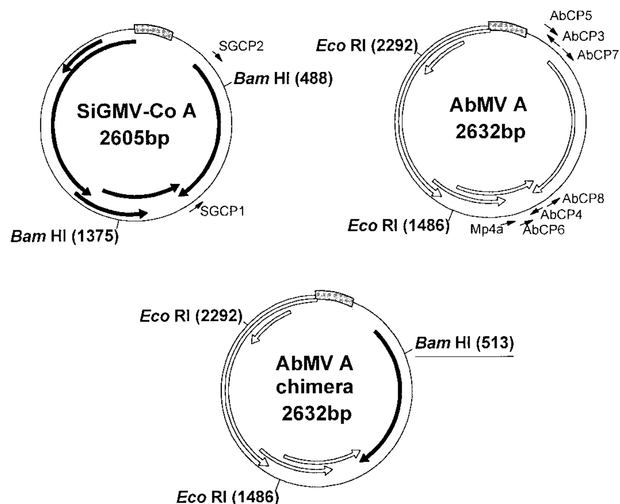

FIG. 1. Genomic maps of AbMV, SiGMV-Co, and AbMV chimera. Diagrammatic presentation of SiGMV-Co DNA A, AbMV DNA A, and chimeric AbMV DNA A. Selected restriction sites and the sequence locations are indicated. The additional *Bam*HI site and sequence location in the AbMV chimera is indicated (underlined). Primers used for the cloning and detection of viral DNA are indicated (see text).

sequence (Fig. 1; Höfer *et al.*, 1997). The amplification protocol was 30 cycles of 1 min at 95°, 1 min at 42°, and 1.5 min at 72°. The coat protein PCR product was blunt-end cloned into the AbMV PCR product, containing the whole pBluescript KS+ vector sequence with residual AbMV sequences. The exact insertion and correctness of the replaced coat protein gene was determined by sequencing with primers AbCP5 (5′-GTA GCG CTA AGT TGT TGG G-3′, located at position 297–315 in the AbMV sequence), AbCP6 (5′-GTA ATT AGA GCT GTT CAG-3′, located at position 1196–1179 in the AbMV sequence) and Mp4a (5′-CCT GAA CTT CCA AGT TTG GAC GAC-3′, located at position 1314–1291 in the AbMV sequence) (Fig.1).

A partial repeat of chimeric AbMV A was produced by cloning of a *Pst*I (1554)–*Hin*cII (457) fragment of AbMV A into pBluescript KS+ and subsequent introduction of full-length chimeric AbMV A into the unique *Pst*I site. The partial repeat was transferred as an *Xba*I–*Kpn*I fragment into pBin19 (AbMV chimera). Clones were mobilized into *Agrobacterium tumefaciens* LBA4404 by triparental mating as described by Ditta *et al.* (1980).

Inoculation of plants and characterization of viral DNA forms

All virus constructs were agroinoculated as described previously (Stanley *et al.*, 1990). Total cellular nucleic acids were extracted 14 days postinoculation from systemically infected leaves as described by Frischmuth and Stanley (1991). Samples were analyzed either by agarose gel electrophoresis or PCR. Samples containing 5 or 10 μg total nucleic acids were

analyzed on agarose gels in 0.5× TBE buffer and nucleic acids were transferred to nylon membranes. Viral DNA was detected by hybridization with digoxigenin (Boehringer Mannheim) or radioactive [α-^{32}P]dCTP-labeled probes prepared by random priming. DNA components were specifically detected using an *Eco*RV (564)–*Pst*I (2479) fragment of SiGMV-Co DNA A, an *Sst*I (1045)–*Sst*I (2552) fragment of SiGMV-Co DNA B, a *Pst*I (1554)–*Eco*RI (2292) fragment of AbMV DNA A or a *Sal*I (658)–*Sal*I (1802) fragment of AbMV DNA B. Because of the close relationship between AbMV and SiGMV-Co (81.9% in DNA A and 70.7% in DNA B: nucleotide homology) the labeled probes cross-hybridize.

For PCR analysis, 1 μl of total nucleic acids sample was used as template. The primer pair SGCP1 and SGCP2 (see above, Fig. 1) allows a specific amplification of the coat protein gene of SiGMV-Co. For specific amplification of AbMV coat protein the primers AbCP7 (5′-CAA AAT GCC TAA GCG CGA TCT CCC-3′, position 356–379) and AbCP8 (5′-TTT ATT AAT TCA TGA GCG AAT C-3′, position 1117–1096) were used. The AbMV gene was amplified by 30 cycles of 1 min at 95°, 1 min at 42°, and 1.5 min at 72°.

Transmission of viruses by *B. tabaci* and analysis of virus acquisition

A colony of the B-biotype of *B. tabaci*, collected from Florida in 1990 (Bedford *et al.*, 1994), was maintained on *Solanum nigrum* in cages at 25° with 16 hr light photoperiod under fluorescent light. Several hundred whiteflies were fed on infected plants for 24 hr, 48 hr, 72 hr, and 2 weeks before being transferred to uninfected seedlings (20–50 insects per plant). After 1, 2, 3, 4, and 7 days the insects were removed and the plants were fumigated with a carbamate-based insecticide. Plants were analyzed for transmission by PCR and Southern blotting as described above.

To examine the acquisition of viruses by *B. tabaci*, samples of 10 whiteflies were harvested. Total nucleic acids were extracted by grinding each group of whiteflies in 200 μl of extraction buffer (35 m*M* Tris–HCl, (pH 8.0), 0.7 m*M* $CaCl_2$, 50 μg Proteinase K, 0.5% SDS), incubating for 2 hr at 65°, phenol/chloroform (1/1: v/v) treatment, and ethanol precipitation. Samples were analyzed either by agarose gel electrophoresis or PCR. Samples containing nucleic acid equivalent to the amount from four whiteflies were analyzed on agarose gels in 0.5× TBE buffer and nucleic acids were transferred to nylon membranes and probed as described above. For PCR analysis the coat protein genes of SiGMV-Co and AbMV were amplified with primers SGCP1, SGCP2, AbCP7, and AbCP8 (see above; Fig. 1). Samples containing nucleic acid equivalent to the amount from one whitefly were used for the PCR analysis.

RESULTS

Infectivity of cloned AbMV, SiGMV-Co, and AbMV chimera

The infectivity of SiGMV-Co as well as AbMV in various host plants has been demonstrated previously (Frischmuth *et al.*, 1990, 1993; Höfer *et al.*, 1997). The AbMV coat protein gene was replaced by the equivalent SiGMV-Co gene, without changing the 5′ and 3′ flanking sequence regions, to ensure first a correct expression of the coat protein from the chimera and second that all other genes of AbMV remained intact. The exact site of insertion and sequence of the SiGMV-Co coat protein gene were confirmed by sequencing of the entire coat protein and adjacent regions. The chimeric AbMV DNA A construct was infectious on various host plants after coagroinoculation with AbMV DNA B and the host range and symptoms were identical to those of AbMV (Table 1).

Transmission of AbMV, SiGMV-Co, and AbMV-chimera by *B. tabaci*

No acquisition of AbMV was detected by Southern blotting of whitefly nucleic acid extracts using AbMV-specific probes after 24 hr, 48 hr, 72 hr, and 2 weeks feeding on infected *A. sellovianum* (Fig. 2, lanes 3, 4, 7, and 8), agroinfected *S. rhombifolia* (Fig. 3, lane 3), *Malva parviflora* and *Nicotiana tabacum* cv. Xanthi nc (data not shown). For each acquisition access period and each plant species 4 to 8 samples of 10 whiteflies were analyzed. With PCR we could detect AbMV DNA in three of five samples of 10 whiteflies fed for 48 hr on *A. sellovianum* and in four of six samples of 10 whiteflies fed for 48 hr on *S. rhombifolia* (Fig 2, lane 13). Following 48-hr inoculation access on various host plants (using 20–50 insects per plant following a 48-hr acquisition access period), it could be deduced that AbMV was not transmissible, but that SiGMV-Co was readily transmissible and the AbMV-chimera, although transmissible, was less efficiently transmitted (Table 2). The plants were assayed 2 weeks postinoculation but AbMV could not be detected by PCR or Southern blot analysis in assay plants which had been exposed to infectious insects (data not shown).

To analyze the retention of AbMV, whiteflies were transferred to uninfected tobacco seedlings for 1, 2, 3, 4, and 7 days after an acquisition access period of 48 hr on infected *A. sellovianum.* By PCR analysis viral DNA was detected at every time point up to 7 days (data not shown). Even after a 7-day inoculation access period, none of the tobacco seedlings developed symptoms and no viral DNA was detected in these plants (data not shown).

In contrast, SiGMV-Co was acquired by *B. tabaci* from *S. rhombifolia* (Fig. 2, lanes 5, 6, 9, 10, and 14, and Fig. 3, lanes 1 and 2) and transmitted to various host plants (Table 2 and Fig. 4, lanes 3 and 5) after an acquisition

TABLE 1

Host Range Analysis of AbMV- and AbMV-Chimera-Cloned Genomic Components Based on Agroinoculation

Virus	Plant species	Infectivity (infected/ inoculated)
AbMV	*Nicotiana benthamiana* Domin	5/6
	N. tabacum L. cv. Xanthi nc	5/6
	Sida rhombifolia L.	2/10
	Malva parviflora L.	6/6
	Phaseolus vulgaris L.	4/6
AbMV chimera[a]	*N. benthamiana*	5/6
	N. tabacum cv. Xanthi nc	6/6
	S. rhombifolia	3/30
	M. parviflora	6/6
	P. vulgaris	3/6

[a] Plants were agroinoculated with AbMV DNA B and chimeric AbMV DNA A.

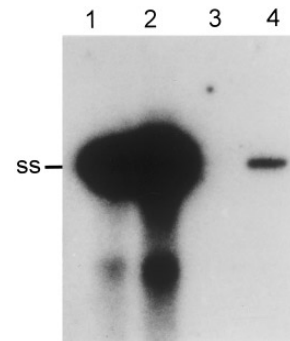

FIG. 3. Southern blot analysis of *B. tabaci* fed on plants infected with AbMV, AbMV chimera, or SiGMV-Co. Nucleic acids of whiteflies fed for 48 hr on *S. rhombifolia* agroinfected with SiGMV-Co (lane 1), *S. rhombifolia* naturally infected with SiGMV-Co (lane 2), *S. rhombifolia* agroinfected with AbMV (lane 3), and *S. rhombifolia* agroinfected with AbMV chimera (lane 4) have been analyzed. The blot was hybridized with a SiGMV-Co DNA A-specific probe. Viral single-stranded (ss) DNA form is indicated.

access time of 48 hr. Retention of virus was observed by PCR and Southern blotting over 7 days (data not shown). The acquisition of SiGMV-Co from agroinoculated *S. rhombifolia* was slightly reduced compared to naturally SiGMV-Co-infected *S. rhombifolia* observed in 10 of 12 replicated experiments (Fig. 3, compare lanes 1 and 2). The transmission frequency of SiGMV-Co to various host plants was similar regardless of whether the source *S. rhombifolia* had been agroinoculated or naturally infected (Table 2).

DNA analysis of whiteflies fed for 48 hr on AbMV-chimera-agroinfected *S. rhombifolia* showed acquisition of this virus by *B. tabaci,* detectable in Southern blots (Fig. 3, lane 4) and by PCR (data not shown). Virus acquisition of the chimera, as judged by Southern blots was much lower than in the case of SiGMV-Co (in 15 of 15 analyses; for example, see Fig. 3, compare lanes 1 and 2 with 4) but was sufficient to transmit the chimeric virus, although at a lower frequency than SiGMV-Co, to *Phaseolus vulgaris* (Top Crop) and *N. tabacum* cv. Xanthi nc (Table 2) after a 48-hr inoculation access period. The symptoms caused by whitefly-transmitted AbMV-chimera were identical to those of agroinoculated bean and tobacco plants. The presence of chimeric AbMV DNA in these whitefly-inoculated plants was confirmed by restriction analysis of nucleic acids (Fig. 4, lane 7). The accumulation of AbMV as well as AbMV-chimera was reduced compared to SiGMV-Co in all plant species tested (Fig. 4).

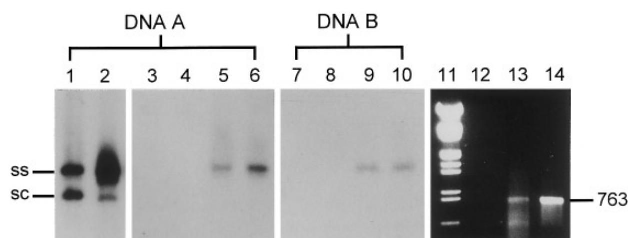

FIG. 2. Southern blot analysis of *B. tabaci* fed on plants infected with AbMV or SiGMV-Co. Nucleic acids of *A. sellovianum* naturally infected with AbMV (lane 1) and *S. rhombifolia* naturally infected with SiGMV-Co (lane 2), as well as nucleic acids of whiteflies which were fed for 48 hr on *A. sellovianum* naturally infected with AbMV (lanes 3, 4, 7, 8, and 13), *S. rhombifolia* naturally infected with SiGMV-Co (lanes 5, 6, 9, 10, and 14), and uninfected *S. rhombifolia* (lane 12) have been analyzed. Samples in lanes 1 to 6 were hybridized with an AbMV DNA A-specific probe and in lanes 7 to 10 with a DNA B-specific probe. Products have been PCR-amplified using primers specific for AbMV coat protein gene sequences in lanes 12 to 14. *Hin*dIII/*Eco*RI-digested lambda DNA was separated in lane 11. Viral supercoiled (sc), single-stranded (ss) DNA forms and size of the PCR amplified coat protein gene (in bp) are indicated.

DISCUSSION

In this paper we have investigated the role of the coat protein in the transmission of a geminivirus by *B. tabaci.* From sequence data, AbMV belongs to the Subgroup III of the Geminiviridae, which are transmitted by whiteflies (Frischmuth *et al.,* 1990). The clone of AbMV was isolated from *A. sellovianum* plants which have been propagated in Europe as stem cuttings for the last century (Regel, 1875). During this long period of maintenance in *A. sellovianum* it is assumed that AbMV lost the ability to be transmitted by *B. tabaci.* Recently Liu *et al.* (1997) showed how insect transmissibility of an ACMV isolate could be lost after 20 passages by mechanical inoculation and suggested that a population had been selected from an original mixture that favored a particular method of transmission. The AbMV isolate, used for these experiments, was not transmitted by *B. tabaci* to various host plants (Table 2). However, small amounts of viral DNA (AbMV) were detected in insects by PCR, but were not detectable in Southern blots. So, although capsids were formed in the plants (virions were purified, data not shown), Southern blots were unable to detect significant amounts of

TABLE 2

Transmission of Sida Golden Mosaic Virus (SiGMV-Co), Abutilon Mosaic Virus (AbMV), and AbMV-Chimera by *Bemisia tabaci*

		Transmission to plant species		
Virus	Source host plant	*Lycopersicon esculentum* Mill.	*Nicotiana tabacum* c.v. Xanthi nc	*Phaseolus vulgaris*
AbMV	*Abutilon sellovianum*[a]	0/6, 0/4, 0/2[b]	0/6, 0/5, 0/6, 0/4, 0/2	0/6, 0/5, 0/6, 0/4, 0/2
	Malva parviflora[c]	0/6, 0/6	0/6	0/6, 0/4
	N. tabacum cv. Xanthi nc[c]	0/9, 0/4	0/4, 0/4	0/6
	Sida rhombifolia[c]	0/4, 0/6	0/4, 0/6	0/4, 0/6
SiGMV-Co	*S. rhombifolia*[d]	4/4, 2/4	6/6, 5/5, 5/6	6/6, 5/5
	S. rhombifolia[e]	n.d	2/2, 6/6, 4/4	6/6, 4/4, 4/4
AbMV chimera[f]	*S. rhombifolia*	n.d.	1/6, 1/4, 0/6	2/4, 0/6, 1/2

[a] *A. sellovianum* plants were original plants from which AbMV was cloned.
[b] Number of plants infected/whitefly-inoculated. Infectivity ratio of different experiments are separated by commas. Transmission was tested using groups of 20–50 insects, after a 48-hr acquisition access period followed by a 48-hr inoculation access period on tomato, tobacco, or beans.
[c] Plants were agroinoculated with AbMV.
[d] *S. rhombifolia* plants were original plants from which SiGMV-Co was cloned.
[e] Plants were agroinoculated with SiGMV-Co.
[f] *S. rhombifolia* plants were agroinoculated with AbMV DNA B and chimeric AbMV DNA A.

single-stranded DNA in the insects which would indicate acquisition of virions. As PCR is more sensitive it may amplify from replicative viral DNA which is only ingested but not acquired by the insects. However, viral DNA was retained within the whitefly over a period of 7 days, which suggests that the DNA was in a stable form, perhaps as a nucleic acid–protein complex. This stable form may be taken up but not transmitted because it was not encapsidated. There is a possibility that virions are acquired, but in numbers too low to detect by Southern blotting. However, this is unlikely, as there is every reason to believe that statistically even a few virions would be transmitted during experiments that used more than 10,000 whiteflies, including many thousand that had been bred on AbMV-infected plants (data not shown). Similar results have been observed for the vector nontransmissible Hawaii isolate of AbMV, where PCR was used to detect virus DNA 7 days after feeding (Wu *et al.,* 1996). In contrast, SiGMV-Co, which was recently isolated from *S. rhombifolia* originating from Costa Rica (Höfer *et al.,* 1997), was readily acquired and transmitted by *B. tabaci* (Table 2). SiGMV-Co was detectable by PCR and Southern blot analysis of insects which showed accumulation of ssDNA.

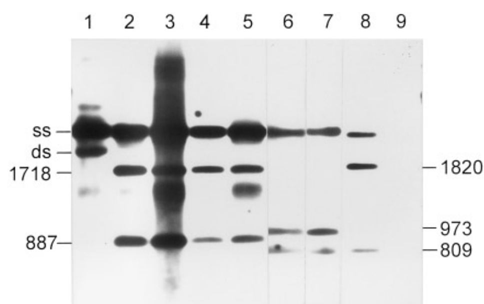

FIG.4. Southern blot analysis of plants infected with AbMV, AbMV chimera, or SiGMV-Co. Nucleic acids of plants infected with SiGMV-Co (lanes 1–5), AbMV chimera (lanes 6 and 7), and AbMV (lane 8) have been analyzed. Samples were either undigested (lane 1) or double-digested with *Bam*HI and *Eco*RI (lanes 2–9). Nucleic acids were extracted from *S. rhombifolia* naturally infected with SiGMV-Co (lanes 1 and 2) and agroinoculated with SiGMV-Co (lane 4), AbMV chimera (lane 6), and AbMV (lane 8). Nucleic acids were extracted from whitefly-inoculated *P. vulgaris* (lanes 3 and 5) and *N. tabacum* cv. Xanthi (lane 7). Prior to inoculation, whiteflies were fed on *S. rhombifolia* either naturally infected with SiGMV-Co (lane 3), agroinfected with SiGMV-Co (lane 5), or agroinoculated with AbMV chimera (lane 7). Nucleic acids of a noninoculated *P. vulgaris* plant were analyzed in lane 9. 5-μg nucleic acids were analyzed in lanes 1 to 5 and 10-μg in lanes 6 to 9. The blot was exposed for 30 min (lanes 1-5), 3 hr (lanes 7–9), or 4 hr (lane 6). The blot was hybridized with a SiGMV-Co DNA A-specific probe. Viral double-stranded (ds) and single-stranded (ss) DNA forms are indicated. Fragment sizes are indicated in bp and correspond to the predicted sizes calculated from the sequences.

The coat protein of geminiviruses is essential for acquisition (Azzam *et al.,* 1994; Liu *et al.,* 1997) and plays a major role in insect specificity (Briddon *et al.,* 1990). We replaced the coat protein gene of the whitefly nontransmissible AbMV isolate with that of the relatively closely related (nucleotide homology: 82% in DNA A and 71% in DNA B) whitefly-transmissible SiGMV-Co without changing adjacent sequences. This exchange was sufficient to produce a whitefly-transmissible chimeric AbMV (Table 2). The acquisition of chimeric AbMV was reduced compared to SiGMV-Co (Fig. 3). This difference in accumulation of viral AbMV-chimera and SiGMV-Co DNA was also observed in infected plants (Fig. 4). The difference in the amount of acquired chimeric virus and SiGMV-Co might be due to either reduced replication ability of chimeric AbMV in various host plants, different tissue

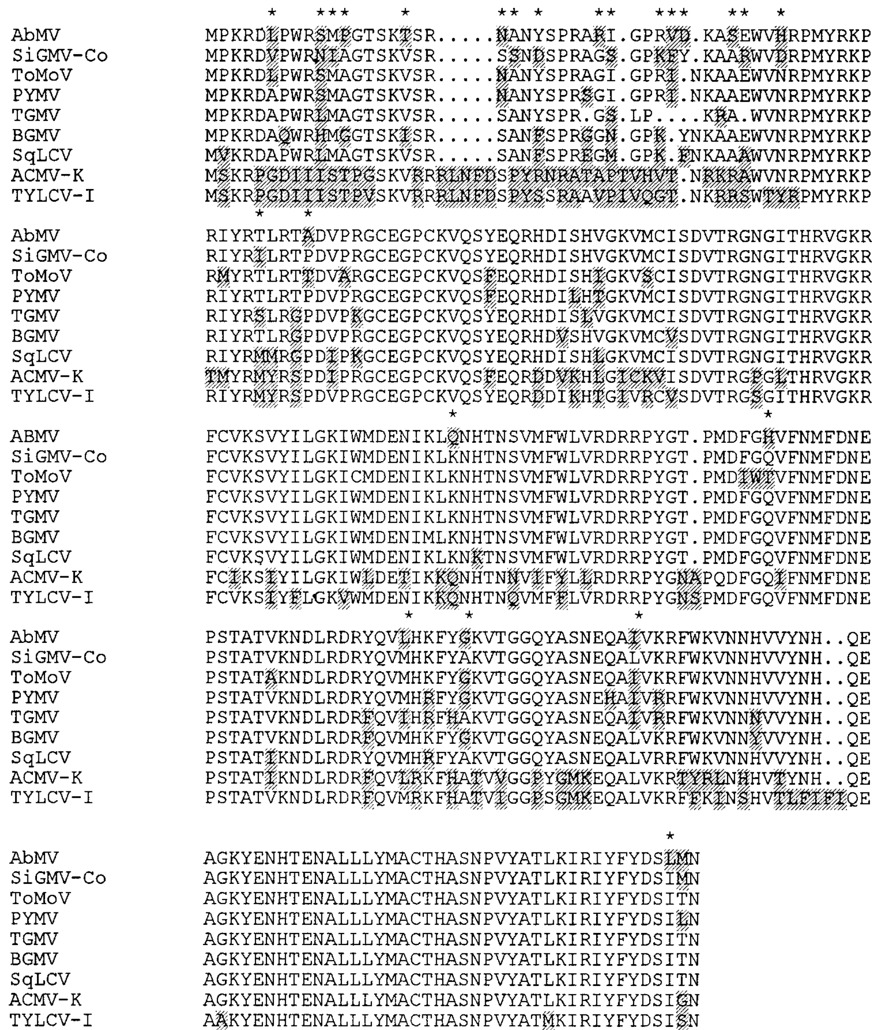

FIG. 5. Alignment of the predicted amino acid sequences of the coat protein of whitefly-transmitted geminiviruses. New World viruses are: AbMV (sequence corrected according to S. Frischmuth, personal communication), SiGMV-Co, tomato mottle virus (ToMoV), bean golden mosaic virus (BGMV), squash leaf curl virus (SqLCV), potato yellow mosaic virus (PYMV), tomato golden mosaic virus (TGMV). Old World viruses are African cassava mosaic virus-Kenyan strain (ACMV-K) and tomato yellow leaf curl virus-Israel strain (TYLCV-I). Sequence data were extracted from the EMBL Database. Amino acids which differ between AbMV and SiGMV-Co are identified (*). Amino acids which are different from the consensus are highlighted.

tropism, or reduced stability of virions. Mutations in the coat protein are reputed to cause a marked decrease in ssDNA in plants (Stanley and Townsend, 1986; Etessami *et al.*, 1989; Padidam *et al.*, 1996). Since the coat protein of SiGMV-Co may not complement all the functions of the AbMV coat protein, this could result in a reduced accumulation of chimeric AbMV within a plant and subsequently in reduced acquisition by *B. tabaci.* A similar observation was reported after replacement of the ACMV coat protein gene with that of BCTV, where leafhoppers could not acquire the chimeric ACMV from infected plants but transmitted the virus after injection of insects with purified virus or following membrane feeding (Briddon *et al.*, 1990). It has also been reported for SqLCV that the coat protein is able to mask the phenotypes of certain BV1 mutants (Ingham *et al*, 1995), although it has been shown that the coat protein of bipartite geminiviruses is dispensable for systemic infection (Stanley and Townsend, 1986; Etessami *et al*, 1989; Gardiner *et al.*, 1988; Azzam *et al*, 1994). This also indicates a possible role for the coat protein in the systemic viral movement pathway (Pooma *et al.*, 1996; Briddon *et al.*, 1989; Boulton *et al.*, 1989). Recent experiments with whitefly-transmissible and whitefly-non-transmissible clones of ACMV showed that the movement protein(s) are also implicated in the acquisition process (Liu *et al.*, 1997). It might be possible that the coat protein functions in such a way as to direct the virus to certain tissues in the plant that are important during the feeding of the vector. The reduced acquisition and transmission of the chimeric AbMV might suggest that other viral genes are involved in these processes as discussed above. However, these data con-

firm that the coat protein plays a key role in transmission of geminiviruses by *B. tabaci.*

Comparison between the coat protein amino acid sequences of AbMV and SiGMV-Co revealed a divergence in the amino terminus and a large area of homology in the carboxy terminus (Fig. 5). The greatest number of amino acid differences is between Old World and New World viruses, but especially in the amino terminus. It cannot be deduced from this comparison which domain of the coat protein, the variable amino terminus, or the relatively conserved carboxy terminus, is responsible for acquisition and transmission of geminiviruses by *B. tabaci.* Even the homologous part shows variations in the primary amino acid sequences. Therefore, single domains of the AbMV coat protein will be replaced by SiGMV-Co counterparts in future experiments to determine the sequence involved in virus–vector interactions.

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