Journal of General Virology (1997), **78**, 1785–1790. Printed in Great Britain

Nucleotide sequence of a new bipartite geminivirus isolated from the common weed *Sida rhombifolia* in Costa Rica

Petra Höfer, Margit Engel, Holger Jeske and Thomas Frischmuth

Biologisches Institut, Abteilung für Molekularbiologie und Virologie der Pflanzen, Universität Stuttgart, Pfaffenwaldring 57, D-70550 Stuttgart, Germany

The nucleotide sequence of infectious clones of a geminivirus from Costa Rica that infects *Sida rhombifolia* was determined. Sida golden mosaic virus (SiGMV-Co) has a bipartite genome (DNAs A and B). Computer analysis showed that the bipartite genome of SiGMV-Co resembles that of other whitefly-transmitted geminiviruses. The DNA A (2605 nt) and DNA B (2587 nt) components have little sequence homology other than within the common region (CR). Analysis of DNAs A and B showed that SiGMV-Co is closely related to bean dwarf mosaic virus (BDMV). SiGMV-Co was introduced via agroinoculation into seven plant species, including tomato and bean.

Introduction

Geminiviruses are serious pathogens of diverse crops that are widely distributed throughout tropical, subtropical and, to a more limited extent, temperate regions of the world. Members of the geminivirus family have been divided into three subgroups (Murphy *et al.*, 1995). The majority of group members that infect dicotyledonous plants are whitefly-transmitted and have bipartite genomes (DNAs A and B). DNA A encodes the coat protein as well as proteins required for replication and gene regulation. DNA B is essential for disease production but plays no role in DNA replication. The two gene products encoded by DNA B are involved in spread of the virus throughout the plant, development of symptoms and in determining the host range of the virus (Lazarowitz, 1992; Timmermans *et al.*, 1994; Noueiry *et al.*, 1994; Ingham *et al.*, 1995).

Diseases caused by whitefly-transmitted geminiviruses impose important constraints on crop production in tropical and subtropical regions. In some cases the incidence of geminiviruses can be so severe that certain economically important crops are not grown, e.g. African cassava mosaic virus (ACMV; Stanley & Gay, 1983) in cassava (Thresh *et al.*, 1994). Until recently, tomato and bean plants grown in the western hemisphere were not known to be seriously affected by geminiviruses. However, geminivirus-associated epidemics are currently threatening tomato, bean and pepper production in Central America (e.g. Mexico, Puerto Rico and Costa Rica) and the southern United States (Simone *et al.*, 1990; Hidayat *et al.*, 1993; Torres-Pacheco *et al.*, 1993; Brown *et al.*, 1995).

Sida rhombifolia is a common weed which grows all over Latin America and the southern United States. Often plants are infected with whitefly-transmitted geminiviruses which might be the source of geminivirus-associated epidemics (Gilbertson *et al.*, 1993). In this paper we describe the genome of a new bipartite geminivirus isolated from *S. rhombifolia* collected in Costa Rica.

Author for correspondence: Thomas Frischmuth.
Fax +49 711 685 5096. e-mail thomas.frischmuth@po.uni-stuttgart.de

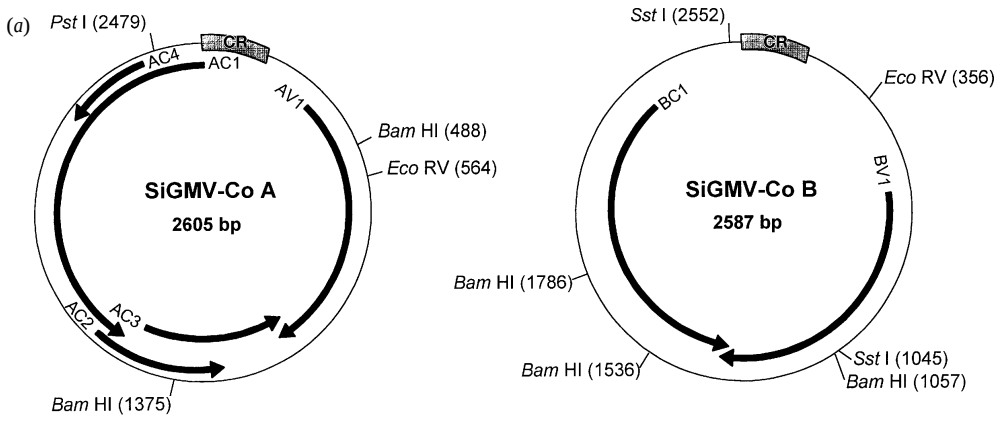
The sequence data reported here are available in the EMBL/GenBank under accession no. X99550 (DNA A) and X99551 (DNA B).

Methods

Virus sources and isolation of double-stranded DNA forms. *S. rhombifolia* plants infected with SiGMV-Co (kindly provided by Peter Markham, John Innes Centre, Norwich, UK) were collected in Costa Rica. The virus was sap-transmitted to *Nicotiana benthamiana* and viral double-stranded DNA was isolated from infected plants as described previously (Frischmuth & Stanley, 1991).

Cloning and sequencing of SiGMV-Co DNAs A and B. 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 sequences of DNAs A and B were determined by either the dideoxynucleotide chain termination method (Pharmacia) with [α-^{35}S]dATP or by automatic sequence analysis with the Li-Cor system, according to the manufacturers' instructions. Sequence analyses were performed with WDNASIS, PROSIS and CLUSTAL W computer programs.

For cloning of full-length DNA A the unique *Pst*I site was used. Viral double-stranded DNA was digested with *Pst*I and linearized DNA was cloned into pBluescript KS(−) (Stratagene). Full-length DNA B was



ORF	MW (kD)
AC1	41.0
AC2	14.8
AC3	15.7
AC4	9.3
AV1	29.3

ORF	MW (kD)
BC1	33.3
BV1	29.7

(b)

```
  1
A GGCGGTGGCATTTTTCTAATAAGAGGAAGTACTCTCTTTGG-TTA
              *      ****          **
B GGCGGTGGCATTTTTGTAATAAGAATGGGTACTCTAGTTGGAGTA
  1

A CTCCAGTTGAGCCCTCTCAAACTTGCTGATTCTATTGGAGTATTA
                          *
B CTCCAGTTGAGCCCTCTCAAACTTGCTCATTCTATTGGAGTATTA

A GAGTATCCATATATACTACTAGTCTCAATCTTGGTTTTGATACAC
    *           *  *  **          *
B GAGTA-CCATATATACTACTTGTGTTTATCTTGGTTTTGGTACAC

                                                184
A GTGGCGGCCATCCGTATAATATTACCGGATGGCCGCGC---GGAGTATGC
                                        ***
B GTGGCGGCCATCCGTATAATATTACCGGATGGCCGCGCTTTGGAGTATGC
                                                181
```

Fig. 1. (*a*) Maps of the SiGMV-Co genomic A and B components. The solid arrows define the position of the open reading frames (C for complementary and V for virus sense). The common region (CR) between DNAs A and B is indicated by a shaded box. The positions of selected restriction endonuclease sites are indicated. The calculated molecular masses (MW) of putative proteins encoded by DNA A and B ORFs are shown below the maps. (*b*) Nucleotide sequence comparison of the common region between DNA A (A) and B (B). Nucleotide mismatches are indicated by asterisks and an inverted repeat sequence able to form a hairpin–loop structure by arrows above the sequence.

cloned as an *Eco*RV fragment. Firstly, an *Sst*I fragment, containing the unique *Eco*RV site of DNA B, was cloned into pBluescript KS(−) and sequences adjacent to the *Eco*RV site were determined in order to design primers for PCR amplification. Full-length DNA B was produced with the primer pair SGMV8 (5′ AGC GAATTC *GAT ATC* AAT TCA ATT TTG AAT TAT TG 3′) and SGMV9 (5′ ATC GAATTC *GAT ATC* GCG CCG ACA TAA AAG G 3′) introducing an extra *Eco*RI site (underlined) at the ends of the PCR product. The PCR fragment was cloned into the *Eco*RI site of pBluescript KS(−) and full-length DNA B could be liberated by *Eco*RV digestion (*Eco*RV site in italics).

A partial repeat of SiGMV-Co DNA A was produced by cloning a *Bam*HI–*Pst*I fragment into pBluescript KS(−) and subsequent introduction of full-length DNA A into the unique *Pst*I site. The SiGMV-Co DNA B partial repeat consisted of an *Eco*RV–*Sst*I fragment and the full-length *Eco*RV DNA B fragment. The partial DNA A repeat was transferred as an *Xba*I–*Kpn*I fragment into pBin19. The partial repeat of DNA B was first amplified with the primer SGMV10 (5′ AGC GAATTC GGC GAA TTG GAG CTC 3′) and the reverse primer of pBluescript KS(−). With primer SGMV10 an additional *Eco*RI site (underlined) was added, allowing transfer of the partial DNA B repeat as a *Hin*dIII–*Eco*RI fragment into pBin19. Clones were mobilized into *Agrobacterium tumefaciens* LBA4404 by triparental mating (Stanley *et al.*, 1990).

■ **Inoculation of plants and characterization of viral DNA forms.** Cloned genomic components were introduced into plants by agroinoculation (Stanley *et al.*, 1990).

Total cellular nucleic acids were extracted 14 days post-inoculation from systemically infected leaves as described by Frischmuth & Stanley (1991). Samples were analysed either by agarose gel electrophoresis or by the PCR method. Samples containing 5 μg total nucleic acids were

analysed on agarose gels in 0·5 × TBE buffer and nucleic acids were transferred to nylon membranes. Viral DNA was detected by hybridization with digoxigenin-labelled probes prepared by random priming according to the manufacturer's recommendations (Boehringer Mannheim). DNA components were specifically detected using an *Eco*RV (564)–*Pst*I (2479) fragment of DNA A and an *Sst*I (1045)–*Sst*I (2552) fragment of DNA B.

For PCR analysis primers SGCP1 (5′ TTT ATT AAT TCA TTA TCG 3′) and SGCP2 (5′ CAA AAT GCC TAA GCG CGA 3′) were used. This primer pair allows a specific amplification of the coat protein gene of SiGMV-Co. PCR fragments were analysed on agarose gels in 0·5 × TBE.

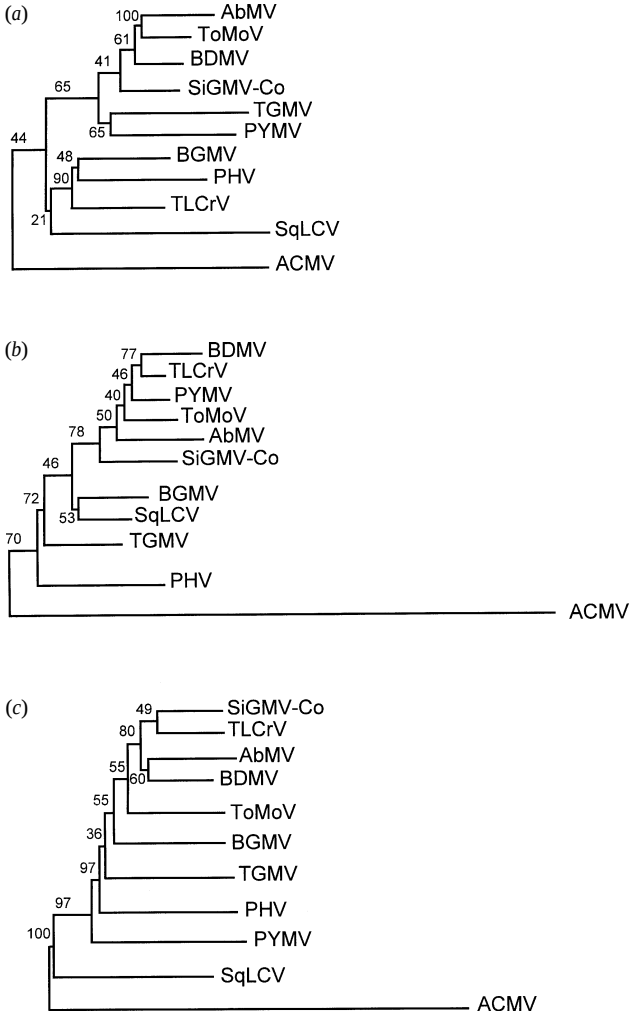


Fig. 2. Phylogenetic trees obtained from aligned CR nucleotide sequences (*a*) and coat protein (*b*) as well as BV1 (*c*) amino acid sequences of SiGMV-Co, BDMV (Hidayat *et al.*, 1993), AbMV (Frischmuth *et al.*, 1990; amended according to S. Frischmuth, personal communication), ToMoV (Abouzid *et al.*, 1992), potato yellow mosaic virus (PYMV; Coutts *et al.*, 1991), BGMV (Howarth *et al.*, 1985), tomato golden mosaic virus (TGMV; Hamilton *et al.*, 1984; amended according to von Arnim & Stanley, 1992), squash leaf curl virus (SqLCV; Lazarowitz & Lazdins, 1991), PHV (Torres-Pacheco *et al.*, 1993), TLCrV (Paplomatas *et al.*, 1994) and ACMV (Stanley & Gay, 1983). The dendrograms were calculated using the neighbour-joining and bootstrap (1000 replications) options of CLUSTAL W. Vertical branches are arbitrary, horizontal branches are proportional to calculated mutation distances. Numbers at nodes indicate percentage bootstrap scores.

Results and Discussion

The increasing incidence of geminiviruses threatens the production of tomatoes, beans and peppers in Latin America (Simone *et al.*, 1990; Hidayat *et al.*, 1993; Torres-Pacheco *et al.*, 1993; Brown *et al.*, 1995). A number of bipartite geminiviruses from infected tomatoes, beans and peppers have been analysed (Morales *et al.*, 1990; Gilbertson *et al.*, 1991; Abouzid *et al.*, 1992; Hidayat *et al.*, 1993; Torres-Pacheco *et al.*, 1993; Paplomatas *et al.*, 1994).

S. rhombifolia grows as a common weed in Central America and plants are often infected with whitefly-transmitted viruses (Bird, 1958). To confirm the presence of a geminivirus in ornamental *S. rhombifolia*, plants were collected in Costa Rica and a bipartite geminivirus was isolated. The nucleotide sequences of the full-length genomic components A and B were determined. The sequence data are available in the EMBL/GenBank under accession no. X99550 (DNA A) and X99551 (DNA B). The sequences are numbered beginning at the first nucleotide of the common region (CR) between DNAs A and B (Fig. 1*b*). The genomic structure of SiGMV-Co, deduced from the nucleotide sequences, resembles that of other bipartite geminiviruses (Fig. 1*a*). DNA A encodes five and DNA B for two putative genes (Fig. 1*a*). The sequence of the CR between DNAs A and B is almost identical over

Table 1. Comparison of amino acid sequences encoded by putative genes of SiGMV-Co with 10 other bipartite geminiviruses

The computer program PROSIS was used to compare sequences.

	Percentage identity to SiGMV-Co						
Virus*	AC1	AC2	AC3	AC4	AV1	BC1	BV1
AbMV	82·8	77·5	81·8	67·1	90·4	89·4	76·6
ACMV	64·0	49·6	51·5	42·4	77·4	39·5	31·3
BDMV	89·2	82·9	79·5	72·9	92·0	92·8	80·5
BGMV	74·0	71·3	73·5	42·4	91·2	79·5	75·9
PHV	70·4	57·7	62·1	69·0	88·0	82·6	71·1
PYMV	77·6	80·5	80·3	60·0	90·8	87·7	67·4
SqLCV	62·5	68·5	72·7	24·1	91·6	78·8	64·2
TGMV	76·6	71·3	76·5	55·3	90·4	81·2	70·7
ToMoV	82·9	75·8	79·5	62·4	90·4	86·0	76·7
TLCrV†	–	–	–	–	93·6	–	82·8‡
							82·4¶

* Sources of sequences are given in Fig. 2.

† The AV1 and BV1 gene sequences for TLCrV are from Paplomatas *et al.* (1994).

‡ Amino acid sequence of BV1 of clone pBMX9B (Paplomatas *et al.*, 1994).

¶ Amino acid sequence of BV1 of clone pTMX44B (Paplomatas *et al.*, 1994).

Table 2. Host range of SiGMV-Co based upon agroinoculation experiments.

Plant species	Infectivity (infected/inoculated)	Symptoms
Nicotiana benthamiana D.	9/9	Severe stunting, leaf curling
Nicotiana tabacum L. cv. Xanthi	6/6	Severe stunting, leaf curling
Nicotiana tabacum L. cv. Samsun	6/6	Severe stunting, leaf curling
Datura stramonium L.	4/6	Severe stunting, leaf curling, mosaic
Lycopersicon esculentum M.	2/6	Stunting, leaf curling
Phaseolus vulgaris L.	6/6	Severe stunting, leaf curling
Sida rhombifolia L.	3/20	Initial stunting and leaf curling*, mosaic
Malva parviflora L.	9/9	Severe stunting, leaf curling, mosaic

* Following agroinoculation of *S. rhombifolia*, plants were initially stunted and leaves showed curling and mosaic. The stunting and leaf curling symptoms disappeared 4 weeks later and only the mosaic on leaves remained.

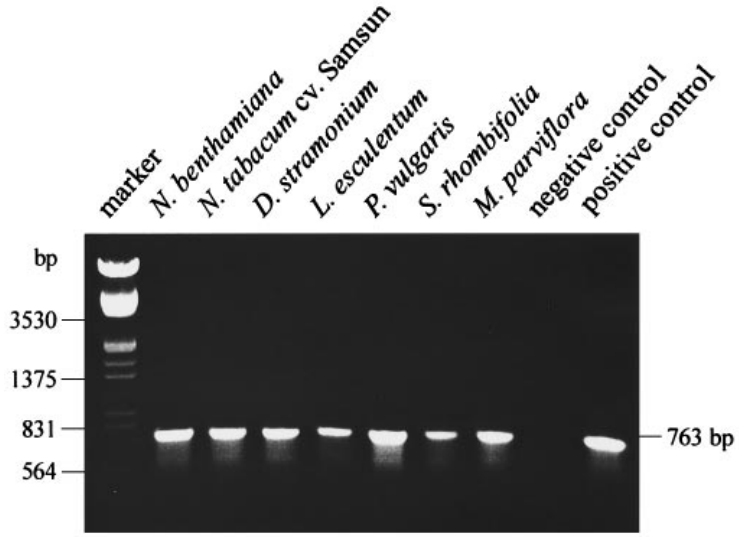


Fig. 3. PCR analysis of SiGMV-Co agroinoculated *Nicotiana benthamiana*, *N. tabacum* cv. Samsun, *Datura stramonium*, *Lycopersicon esculentum*, *Phaseolus vulgaris*, *Sida rhombifolia*, *Malva parviflora* and uninfected *N. benthamiana* (negative control). SiGMV-Co DNA A was used as template for PCR (positive control). The molecular mass standard was *Eco*RI/*Hin*dIII digested λ-DNA (marker). Sizes are indicated in bp.

approximately 180 nucleotides (Fig. 1*b*). The CR contains a sequence which is capable of producing a hairpin–loop structure (Fig. 1*b*). This structure is identical to that found in other geminiviruses (Lazarowitz, 1992).

Comparison of the sequences of SiGMV-Co DNAs A and B with those of other bipartite geminiviruses revealed a close relationship between geminiviruses from the New World and less homology with ACMV from Africa. Phylogenetic analysis of the CR sequences showed that SiGMV-Co clusters with bean dwarf mosaic virus (BDMV) isolated from beans in Colombia, tomato mottle virus (ToMoV; Abouzid *et al.*, 1992) isolated from infected tomato plants in Florida (USA) and Abutilon mosaic virus (AbMV; Frischmuth *et al.*, 1990) (Fig. 2*a*). The infected *Abutilon* plants were probably from the West Indies (Regel, 1875).

A comparison of amino acid sequences encoded by single ORFs confirmed the close relationship between SiGMV-Co and BDMV (Table 1). The amino acid sequences of the coat protein and the BV1 protein of tomato leaf crumple virus (TLCrV; Paplomatas *et al.*, 1994) were also included in this analysis and for all the viruses examined, these two TLCrV sequences showed the greatest identity to their counterparts in SiGMV-Co (Table 1). In the multiple alignment analysis of these amino acid sequences, SiGMV-Co clusters with BDMV, AbMV, ToMoV and TLCrV (Fig. 2*b, c*). In contrast, in the CR analysis (Fig. 2*a*) TLCrV clusters with pepper huasteco virus (PHV; Torres-Pacheco *et al.*, 1993) and bean golden mosaic virus (BGMV; Howarth *et al.*, 1985). This difference for TLCrV may reflect its origin because PHV as well as TLCrV was isolated in Mexico (Torres-Pacheco *et al.*, 1993; Paplomatas *et al.*, 1994). A noticeable feature of the amino acid sequence analysis was the low identity between AC4 in SiGMV-Co compared to the other viruses studied (Table 1). This diversity might be due to the fact that no function of this gene has been

found *in planta* (Elmer *et al.*, 1988; Etessami *et al.*, 1991), although protoplast experiments have indicated that AC4 may be involved in the regulation of AC1 (Gröning *et al.*, 1994). Taken together, results show that SiGMV-Co is most closely related to bipartite geminiviruses from Central America.

Utilizing the agroinoculation method we were able to infect various plant species with SiGMV-Co (Table 2). The general symptoms in all infected plant species were stunting of the plant and leaf curling. Some plant species, e.g. *Datura stramonium*, *Malva parviflora* and *S. rhombifolia*, also exhibited yellow-green mosaic on infected leaves (Table 2). Symptomatic plants were analysed by Southern blotting (data not shown) and PCR (Fig. 3). Viral DNA was detected in all infected plants (Fig. 3). With the exception of *S. rhombifolia*, all other plants were inhibited in their growth for several weeks post-inoculation. The first symptoms of infection (stunting, leaf curling and mosaic) on the leaves of inoculated *S. rhombifolia* plants resembled those on other plant species. However, approximately 4 weeks later the growth inhibition and leaf curling symptoms disappeared and only the mosaic remained (Table 2). After this period of time symptoms of agroinoculated plants were indistinguishable from those of the original *S. rhombifolia* plants.

We have shown that SiGMV-Co infects a number of plant species, including tomato and bean. Therefore, infected *S. rhombifolia* plants might serve as a natural host reservoir for geminiviruses from which crop plants could be infected. That ornamental *Sida* or *Abutilon* plants might serve as natural host reservoirs for geminiviruses has also been proposed by Gilbertson *et al.* (1993). *S. rhombifolia* is a common weed in Central America and close monitoring of *S. rhombifolia* plants for infection could lead to a reduced incidence of geminiviruses in crops.

SiGMV-Co clones were held according the Gentechnik Gesetz (Licence no. 76–14/8829.02/Uni.S.01.01–5 and 76–14/8829.02/Uni.S.01.04–2). The work was supported by a DFG grant to T.F. (Fr. 1122/1–1).

References

Abouzid, A. M., Polston, J. E. & Hiebert, E. (1992). The nucleotide sequence of tomato mottle virus, a new geminivirus isolated from tomatoes in Florida. *Journal of General Virology* **73**, 3225–3229.

Bird, J. (1958). Infectious chlorosis of *Sida carpinifolia* in Puerto Rico. Technical Paper, University of Puerto Rico Agricultural Experimental Station 26.

Brown, J. K., Bird, J., Banks, G., Sosa, M., Kiesler, K., Cobrera, I. & Fornaris, G. (1995). First report of an epidemic in tomato caused by two whitefly-transmitted geminiviruses in Puerto Rico. *Plant Disease* **79**, 1250.

Coutts, R. H. A., Coffin, R. S., Roberts, E. J. F. & Hamilton, W. D. O. (1991). The nucleotide sequence of the infectious cloned DNA components of potato yellow mosaic virus. *Journal of General Virology* **72**, 1515–1520.

Elmer, J. S., Brand, L., Sunter, G., Gardiner, W. E., Bisaro, D. M. & Rogers, S. G. (1988). Genetic analysis of the tomato golden mosaic virus. II. The product of the AL1 coding sequence is required for replication. *Nucleic Acids Research* **16**, 7043–7060.

Etessami, P., Saunders, K., Watts, J. & Stanley, J. (1991). Mutational analysis of complementary-sense genes of African cassava mosaic virus DNA A. *Journal of General Virology* **72**, 1005–1012.

Frischmuth, T. & Stanley, J. (1991). African cassava mosaic virus DI DNA interferes with the replication of both genomic components. *Virology* **18**, 539–544.

Frischmuth, T., Zimmat, G. & Jeske, H. (1990). The nucleotide sequence of Abutilon mosaic virus reveals prokaryotic as well as eukaryotic features. *Virology* **178**, 461–468.

Gilbertson, R. L., Faria, J. C., Hanson, S. F., Morales, F. J., Ahlquist, P., Maxwell, D. P. & Russell, D. R. (1991). Cloning of the complete DNA genomes of four bean-infecting geminiviruses and determining their infectivity by electric discharge particle acceleration. *Phytopathology* **81**, 980–985.

Gilbertson, R. L., Hidayat, S. H., Paplomatas, E. J., Rojas, M. R., Hou, Y.-M. & Maxwell, D. P. (1993). Pseudorecombination between infectious cloned DNA components of tomato mottle and bean dwarf mosaic geminivirus. *Journal of General Virology* **74**, 23–31.

Gröning, B. R., Hayes, R. J. & Buck, K. W. (1994). Simultaneous regulation of tomato golden mosaic virus coat protein and AL1 gene expression: expression of the AL4 gene may contribute to suppression of the AL1 gene. *Journal of General Virology* **75**, 721–726.

Hamilton, W. D. O., Stein, V. E., Coutts, R. H. A. & Buck, K. W. (1984). Complete nucleotide sequence of the infectious cloned DNA components of tomato golden mosaic virus: potential coding regions and regulatory sequences. *EMBO Journal* **3**, 2197–2205.

Hidayat, S. H., Gilbertson, R. L., Hanson, S. F., Morales, F. J., Ahlquist, P., Russell, D. R. & Maxwell, D. P. (1993). Complete nucleotide sequences of the infectious cloned DNAs of bean dwarf mosaic geminivirus. *Phytopathology* **83**, 181–187.

Howarth, A. J., Caton, J., Bossert, M. & Goodman, R. M. (1985). Nucleotide sequence of bean golden mosaic virus and a model for gene regulation in geminiviruses. *Proceedings of the National Academy of Sciences, USA* **82**, 3572–3576.

Ingham, D. J., Pascal, E. & Lazarowitz, S. G. (1995). Both bipartite geminiviruses movement proteins define viral host range, but only BL1 determines viral pathogenicity. *Virology* **207**, 191–204.

Lazarowitz, S. G. (1992). Geminiviruses: genome structure and gene function. *Critical Reviews in Plant Science* **11**, 327–349.

Lazarowitz, S. G. & Lazdins, I. B. (1991). Infectivity and complete nucleotide sequence of the cloned genomic components of a bipartite squash leaf curl geminivirus with a broad host range phenotype. *Virology* **180**, 58–69.

Morales, F., Niessen, A., Ramirez, B. & Castano, M. (1990). Isolation and partial characterisation of a geminivirus causing bean dwarf mosaic. *Phytopathology* **80**, 96–101.

Murphy, F. A., Fauquet, C. M., Bishop, D. H. L., Ghabrial, S. A., Jarvis, A. W., Martelli, G. P., Mayo, M. A. & Summers, M. D. (editors) (1995). *Virus Taxonomy. Sixth Report of the International Committee on Taxonomy of Viruses*. Vienna & New York: Springer-Verlag.

Noueiry, A. O., Lucas, W. J. & Gilbertson, R. L. (1994). Two proteins of a plant DNA virus coordinate nuclear and plasmodesmal transport. *Cell* **76**, 925–932.

Paplomatas, E. J., Patel, V. P., Hou, Y. M., Noueiry, A. O. & Gilbertson, R. L. (1994). Molecular characterization of a new sap-transmissible bipartite genome geminivirus infecting tomatoes in Mexico. *Phytopathology* **84**, 1215–1224.

Regel, E. (1875). Neue oder empfehlenswerte Zierpflanzen. *Gartenflora* **24**, 116–117.

Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989). *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Simone, G. W., Brown, J. K., Hiebert, E. & Cullen, R. E. (1990). New geminivirus epidemic in Florida tomatoes and peppers. *Phytopathology* **80**, 1063.

Stanley, J. & Gay, M. (1983). Nucleotide sequence of cassava latent virus DNA. *Nature* **301**, 260–262.

Stanley, J., Frischmuth, T. & Ellwood, S. (1990). Defective viral DNA ameliorates symptoms of geminiviruses infection in transgenic plants. *Proceedings of the National Academy of Sciences, USA* **87**, 6291–6295.

Thresh, J. M., Fargette, D. & Otim-Nape, W. G. (1994). Effects of African cassava mosaic geminivirus on the yield of cassava. *Tropical Science* **34**, 26–42.

Timmermans, M. C. P., Das, O. P. & Messing, J. (1994). Geminiviruses and their uses as extrachromosomal replicons. *Annual Review of Plant Physiology and Plant Molecular Biology* **45**, 79–112.

Torres-Pacheco, I., Garzón-Tiznada, J. A., Herrera-Estrella, L. & Rivera-Bustamante, R. F. (1993). Complete nucleotide sequence of pepper huasteco virus: analysis and comparison with bipartite geminiviruses. *Journal of General Virology* **74**, 2225–2231.

von Arnim, A. & Stanley, J. (1992). Determinants of tomato golden mosaic virus symptom development located on DNA B. *Virology* **186**, 286–293.

Received 8 November 1996; Accepted 24 February 1997