Arch Virol (2008) 153:2149–2155
DOI 10.1007/s00705-008-0238-z

BRIEF REPORT

Agroinfection of cloned Sri Lankan cassava mosaic virus DNA to *Arabidopsis thaliana*, *Nicotiana tabacum* and cassava

Dheeraj Mittal · Basanta Kumar Borah · Indranil Dasgupta

Received: 20 July 2008 / Accepted: 6 October 2008 / Published online: 4 November 2008

Abstract Sri Lankan cassava mosaic virus (SLCMV) is a bipartite begomovirus infecting cassava in India and Sri Lanka. We have used Agrobacterium-mediated inoculation (agroinoculation) of cloned SLCMV DNA to inoculate additional hosts, *Nicotiana tabacum* and Arabidopsis. Although SLCMV infection in these hosts caused stunting, leaf deformation and developmental abnormalities, accumulation levels of viral DNA in the infected plants suggested that this virus was poorly adapted to them. In the natural host, cassava, agroinoculation produced infection at a low frequency. The monopartite nature of SLCMV, reported earlier in *N. benthamiana*, was maintained in the new hosts as well as in cassava.

Cassava mosaic disease (CMD) is one of the major constraints in the cultivation of cassava, an important tropical root crop, in the African continent and India [5, 9]. Several virus species are known to be associated with CMD, all belonging to the family *Geminiviridae*, genus *Begomovirus* [20, 21] and contain two single-stranded DNA components, DNA-A and DNA-B, as their genome. DNA-A encodes proteins related to replication and encapsidation of the viral DNA and control of viral gene expression, whereas DNA-B encodes movement-related proteins. DNA-A and DNA-B share a ~200-bp common region (CR), which carries DNA replication and transcriptional signals. Replication of the viral DNA initiates at a stem-loop structure within the CR carrying the nonanucleotide sequence conserved in all geminiviruses. In India, Sri Lankan cassava mosaic virus (SLCMV) is the most common virus responsible for CMD [4, 12, 15, 19], along with the closely related Indian cassava mosaic virus, ICMV [9, 12, 15, 16, 19].

Although both of the DNA components of SLCMV, when introduced by mechanical inoculation or biolistics, are necessary for infection in the experimental host *Nicotiana benthamiana*, DNA-A alone can cause infection when introduced by agroinoculation. This observation, along with an unexpectedly high overall nucleotide sequence identity of SLCMV DNA-B to ICMV DNA-B suggested that SLCMV DNA-A possesses monopartite characteristics, and its observed bipartite nature in the natural host cassava has been possibly achieved by capturing and suitably modifying ICMV DNA-B to give rise to SLCMV DNA-B [19]. However, the monopartite nature of SLCMV has not been tested on any host other than *N. benthamiana*.

Infectious cloned geminiviral DNAs are powerful tools in the study of viral host range, *trans*-replication of DNA components and genetic determinants of symptom production [19]. Of the three artificial methods used to introduce geminiviral DNA to plant hosts (mechanical rubbing, biolistic delivery and agroinoculation), agroinoculation is considered to be the most efficient. In agroinoculation, the viral DNA is cloned in a binary vector, followed by transfer to *Agrobacterium tumefaciens* and injection of the bacterial suspension into the plant to allow DNA transfer to take place [6]. For agroinoculation, cloned tandemly arranged copies of the viral genome are inserted in a binary vector such that, following transfer to the plant, unit-length viral DNA can be released by replication or recombination [22].

Cloned SLCMV DNA has been shown to be infectious to the experimental host *N. benthamiana* by agroinoculation

D. Mittal · B. K. Borah · I. Dasgupta (✉)
Department of Plant Molecular Biology, University of Delhi South Campus, Benito Juarez Road, New Delhi 110021, India
e-mail: indranil58@yahoo.co.in

[18] and to *N. benthamiana* and cassava by biolistic inoculation [4, 13]. We decided to use the method of agroinoculation to investigate whether SLCMV DNA can be introduced into the model plant Arabidopsis, the natural host cassava and the common laboratory plant *N. tabacum*. Because of the availability of the complete genome sequence of Arabidopsis and a large number of well-characterized mutant lines, a method of infecting Arabidopsis using SLCMV would make available a system to study the molecular interactions between the host and a virus of agricultural importance. Moreover, in the natural host cassava, although a biolistic method for the introduction of cloned geminiviral DNA is available [1, 3], development of agroinfectious clones of SLCMV will open up an additional method of introducing geminiviral DNA, which can be useful in screening large collections of cassava germplasm for CMD resistance.

In this study, we have used cloned SLCMV DNA [4] to test whether it can be introduced to Arabidopsis, *N. tabacum,* and cassava by agroinoculation, in addition to the experimental host *N. benthamiana* reported earlier [18]. We show that agroinoculation can be used to introduce SLCMV DNA to the above hosts, with variable degrees of infectivity. In addition, we demonstrate the monopartite nature of SLCMV in all of the hosts tested.

N. benthamiana plants were grown in pots inside a temperature-controlled room maintained at 24 ± 1°C and a 14/10-h light–dark cycle. *A. thaliana* (L) Heynh. ecotype Columbia (Col) plants were grown under constant illumination (~80 µmol m^{-2}/s), maintained at 22 ± 1°C, in clay pots containing Soilrite (Kelprlite, Bangalore, India; 1:1:1 ratios of vermiculite, perlite and sphagnum moss). Stem cuttings from cassava and seed-germinated *N. tabacum* seedlings were grown in pots inside a glasshouse maintained at 28 ± 2°C with supplementary lighting for a 14/10-h light–dark cycle and at 70% humidity.

Two DNA fragments derived from cloned SLCMV-[Ker20]/Adivaram DNA-A [4], (EMBL accession no. AJ579307) were used to construct the tandem copies of the viral genome: a 1.7-kb *Hin*dIII-*Pst*I fragment (nucleotides 2,502–1,438) and a 1.0-kb *Pst*I-*Hin*dIII fragment (nucleotides 1,439–2,501), the former containing the stem-loop and the latter lacking it. Similarly, two DNA fragments from the cognate DNA-B (AJ579308), a 1.5-kb *Bam*HI-*Hin*dIII fragment (nucleotide positions 1,424–138) and a 1.2-kb *Bam*HI-*Hin*dIII fragment (nucleotide positions 139–1,423) were used, the former containing the stem-loop formation and the latter lacking it. The fragments were cloned in the plasmid cloning vector pBSK+ into *Pst*I-*Hin*dIII and *Bam*HI-*Hin*dIII sites, respectively, followed by ligation to the full length DNA-A in the former case and DNA-B in the latter. The constructs were checked by appropriate restriction digests to ensure that they formed tandem repeats in each case in the same orientation. The tandem repeats representing all four constructs were excised from pBSK vectors and inserted into the binary vector pCambia 2300 [14] using the *Sal*I-*Sac*I sites. The tandemly repeated clones in the binary vector were named SLCMV-A1.7 (A1.7), SLCMV-A1.0 (A1.0), SLCMV-B1.5 (B1.5) and SLCMV-B1.2 (B1.2), of which A1.7 and B1.5 have the stem-loop duplicated. All of the binary vector clones were mobilized into the *Agrobacterium* strain EHA105.

N. benthamiana plants with 5–6 leaves were injected with 20 µl bacterial suspension (in a buffer consisting 10 mM $MgCl_2$, 10 mM MES and 150 µM acetosyringone) at the second and third leaf shoot axis using a fine needle. Cassava and *N. tabacum* plants were inoculated in a similar manner. In Arabidopsis, two leaves were inoculated after injuring the dorsal and ventral leaf surface with a fine needle just prior to the bolting stage.

DNA was isolated as per standard protocol [10, 17] from newly emerged leaves showing symptoms and analyzed by Southern hybridization as described [19]. Viral DNAs were detected using a 1,067-bp *Pst*I-*Hin*dIII fragment of SLCMV DNA-A as probe for detecting DNA-A and a 654-bp *Bgl*II-*Spe*I fragment of SLCMV DNA-B (nucleotide residues 318–972) for detecting DNA-B. The presence of viral DNA was also analyzed by PCR amplification using CP gene-specific primers (forward primer: 5′*AGGATCC*GATCTCTGTCATCAGGG 3′ (nucleotide residues 271–287), Reverse primer: 5′*GGAG CTC*GGGACTGACAGTATTGAG 3′ (nucleotide residues 1,114–1,097), the residues shown in italics being added for ease in cloning, amplifying an 843-bp region of the *cp* gene from DNA-A.

To check whether the clones representing tandem repeats of SLCMV DNA components (A1.7, A1.0, B1.2 and B1.5) show infectivity on the host *N. benthamiana* by agroinoculation, a total of 210 plants were inoculated with various combinations (Table 1) in three independent experiments. Out of 75 plants inoculated with DNA-A alone, symptoms (upward leaf roll, leaf deformity and vein swelling) started appearing in 69 plants by 7 days post-inoculation (dpi), whereas mock-inoculated plants did not show any symptoms, which agreed with earlier reports of agroinoculation of SLCMV DNA [19]. In plants that were inoculated with DNA-A + DNA-B, downward leaf curling was initially observed, but after 15 dpi, newly emerging leaves showed upward leaf roll. Symptomatic plants did not show any signs of recovery. On Southern analysis, all symptomatic plants were seen to accumulate single-stranded, supercoiled and other replicative forms of DNA-A (Fig. 1c). Accumulation of DNA-B was transient and not detectable after 25 dpi. This showed that the cloned DNAs, shown earlier to be infectious on *N. benthamiana* by

Table 1 Infectivity of cloned SLCMV DNAs in *N. benthamiana*, *N. tabacum* Arabidopsis and cassava using agroinoculation. The numbers represent the symptomatic plants/inoculated plants

Inoculated DNA	*N. benthamiana*[1]			*N. tabacum*[2]		Cassava[3]		Arabidopsis[4]		
	a	b	c	a	b	a	b	a	b	c
A1.0	20/20	10/10	5/5	5/5	3/5	5/8	3/4	6/6	NI	NI
A1.0 + B1.2	20/20	10/10	5/5	5/5	2/5	5/8	NI	NI	NI	NI
A1.0 + B1.5	20/20	10/10	5/5	5/5	4/5	4/8	6/6	NI	NI	NI
A1.7	20/20	9/10	5/5	5/5	3/5	5/8	NI	NI	5/5	4/4
A1.7 + B1.2	20/20	10/10	5/5	5/5	2/5	4/8	NI	NI	NI	NI
A1.7 + B1.5	20/20	10/10	5/5	5/5	2/5	4/8	4/4	18/18	5/5	4/4
Mock	0/20	0/10	0/5	0/5	0/5	0/8	0/4	0/10	0/5	0/4

Observations were recorded at the following dpi, 7[1], 30[2], 35[3] and 17[4]

a, *b* and *c* represent results of replicated experiments, *NI* indicates not inoculated

biolistic delivery [4], retain their infectivity when cloned in a tandem-repeat arrangement and introduced to the plant by agroinoculation.

In the case of *N. tabacum*, 46 out of a total of 60 plants inoculated with different combinations started showing symptoms (Table 1) after 25 dpi. By 60 dpi, severe stunting and leaf deformity were observed (Fig. 1a), whereas no symptoms were visible in mock-inoculated plants (Fig. 1b). Symptomatic leaf samples, when analyzed by Southern hybridization, were found to accumulate all of the expected forms of viral DNAs, although to a much lesser extent in comparison to *N. benthamiana* (Fig. 1d). The proportion of *N. tabacum* plants infected was less than with *N. benthamiana* (Table 1), typically approximately 76% as compared to close to 100% in *N. benthamiana*. There was no difference between the symptoms exhibited by plants which had DNA-A only and those having DNA-A + DNA-B.

In the case of cassava, 62 plants were agroinoculated in two independent experiments. Although at 35 dpi, leaf deformity and necrosis (Fig. 2a) were observed in almost 60% of plants inoculated with various combinations (mock-inoculated plants showing no symptoms, Fig. 2b), the symptomatic leaves did not accumulate the viral DNA to detectable levels when checked by Southern hybridization. However, viral DNAs were detectable by PCR (Fig. 2d) in all 42 symptomatic leaves tested, indicating their accumulation at low levels. One plant inoculated with A1.0 only developed mild mosaic symptoms typical of CMD in newly emerging leaves at 45 dpi (Fig. 2c), in which viral DNA was seen to accumulate by Southern hybridization (Fig. 2e). Neither could DNA-B be amplified from that plant, nor did a probe specific for DNA-B hybridize with the plant DNA in Southern analysis, ruling out accidental DNA contamination. This strongly indicated that the infection had resulted from the inoculation of DNA-A alone.

In the case of Arabidopsis, a total of 42 plants were agroinoculated in three sets of experiments with three combinations of SLCMV DNA, namely A1.0, A1.7 and A1.7 + B1.5. At 25 dpi, inoculated plants containing the above DNAs showed the emergence of secondary rosettes, increased branching, abnormal floral meristems, curling of leaves and early dehiscence of the siliques (Fig. 3a, c), but the mock-inoculated plants did not (Fig. 3b). Agroinoculation was also attempted on younger plants, but they did not survive, presumably because of the injuries sustained during the inoculation process. Between the three types of DNAs used for inoculation, there were no differences in the expressed symptoms. All of the symptomatic plants showed accumulation of viral DNA species having low electrophoretic mobility, as assessed by Southern analysis (Fig. 3d). To ascertain the identity of the inoculated molecule in the symptomatic plants, the *cp* gene was amplified from total DNA isolated from a single symptomatic plant, cloned and sequenced. The 843-bp fragment representing nucleotide residues 271–1,114 (*cp* gene) was seen to be identical to SLCMV-[Ker20]/Adivaram DNA-A, the inoculated DNA.

As an extension of infectivity studies of cloned SLCMV DNAs using the biolistic method reported earlier [4, 13, 15], we wanted to know whether agroinoculation can be used as a method to introduce infectious SLCMV DNA back in the natural host cassava, to the common laboratory plant *N. tabacum* and to the model plant Arabidopsis. We show here that cloned SLCMV DNA can be introduced efficiently to Arabidopsis and *N. tabacum*, in addition to *N. benthamiana*, by agroinoculation. In cassava, because the inoculated DNA accumulated to levels much lower than expected in most symptomatic leaves, it can be concluded that the process takes place with low efficiency. The efficiency of infection of SLCMV DNAs used here, (SLCMV-[Ker20]) was seen to be similar to that reported earlier for the Sri Lankan isolate SLCMV-[Col] [19]. Compared to the biolistic method [13],

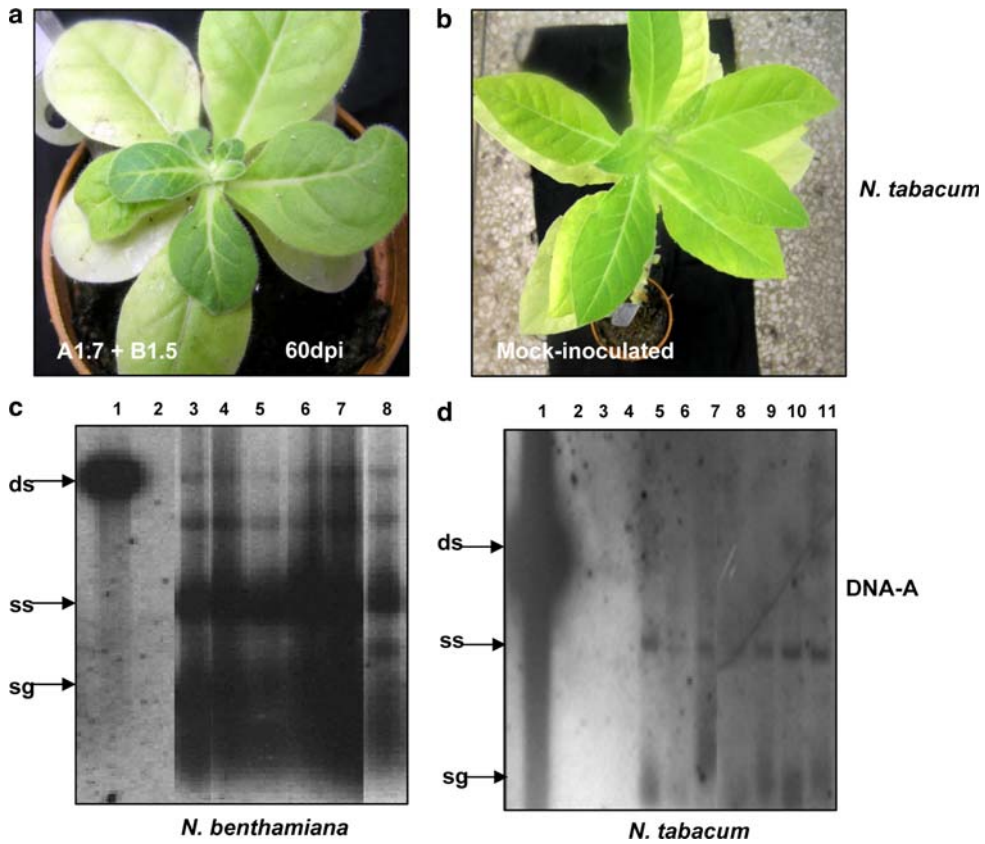

Fig. 1 Infectivity of SLCMV DNAs on *Nicotiana benthamiana* and *N. tabacum*. **a** Symptoms produced following agroinoculation, along with the combination of DNA used and the days elapsed after inoculation, days post-inoculation (*dpi*). **b** Mock-inoculated *N. tabacum* plant showing no symptoms. **c** Southern hybridization analysis of total genomic DNA of SLCMV inoculated and mock-inoculated *N. benthamiana* plants at 30 dpi. *Lane* 1, 5 ng cloned linearized DNA-A as positive control, *lane* 2, DNA from a mock-inoculated plant, *lanes* 3–8, DNA from plants inoculated with A1.7 + B1.5. **d** Southern hybridization analysis of *N. tabacum* plants at 30 dpi. *Lane* 1, 5 ng cloned linearized DNA-A as positive control, *lanes* 2, 3 and 4 DNA from mock-inoculated plants, *lanes* 5–11, DNA from plants inoculated with A1.7 + B1.5. The blots were hybridized with the DNA-A probe representing nucleotide residues 1,439–2,501. The positions of single-stranded (*ss*), double-stranded (*ds*) and subgenomic (*sg*) DNA are indicated by *arrows*

the inoculation efficiencies on *N. benthamiana* by agroinoculation were superior, the respective figures being 60% for biolistics compared to 100% for agroinoculation, thereby establishing agroinoculation as a superior method of inoculation over the biolistic method.

Following agroinoculation-mediated transfer of cloned viral DNA to plants, two viral plus-strand origins of replication present in the same tandemly repeated molecule can result in initiation of DNA synthesis at one origin and termination at the other, leading to replication-mediated release of unit-length viral DNA [22]. The repeat also makes it prone to intra-molecular recombination, resulting in a recombination-mediated release. In contrast, a tandemly repeated molecule containing only a single replication origin can generate replicating viral genomes only by recombination [22]. When the infection efficiencies of constructs with and without the repeated stem-loops were compared, no significant difference was observed (Table 1). Thus, we conclude that replication is not the predominant mechanism deciding the release of unit-length viral DNA following agroinoculation, which would have otherwise shown a difference between the two constructs.

Since the percent infectivities of DNA-A alone were the same to those of DNA-A + DNA-B in all the four hosts tested, it could be concluded that DNA-B was not essential for infectivity of DNA-A. In addition, DNA-B was detected only transiently in *N. benthamiana* plants inoculated with DNA-A and DNA-B. In addition, on co-inoculation, there was a change in the symptoms from initial downward leaf curling, typical of a bipartite begomovirus infection, to upward leaf roll, a symptom associated with monopartite begomoviruses in *N. benthamiana*. This change was particularly seen in emerging leaves late in the infection, reminiscent of a similar observation reported for biolistically inoculated *N. benthamiana* plants with ICMV DNA components [13]. Taken together, the results strongly

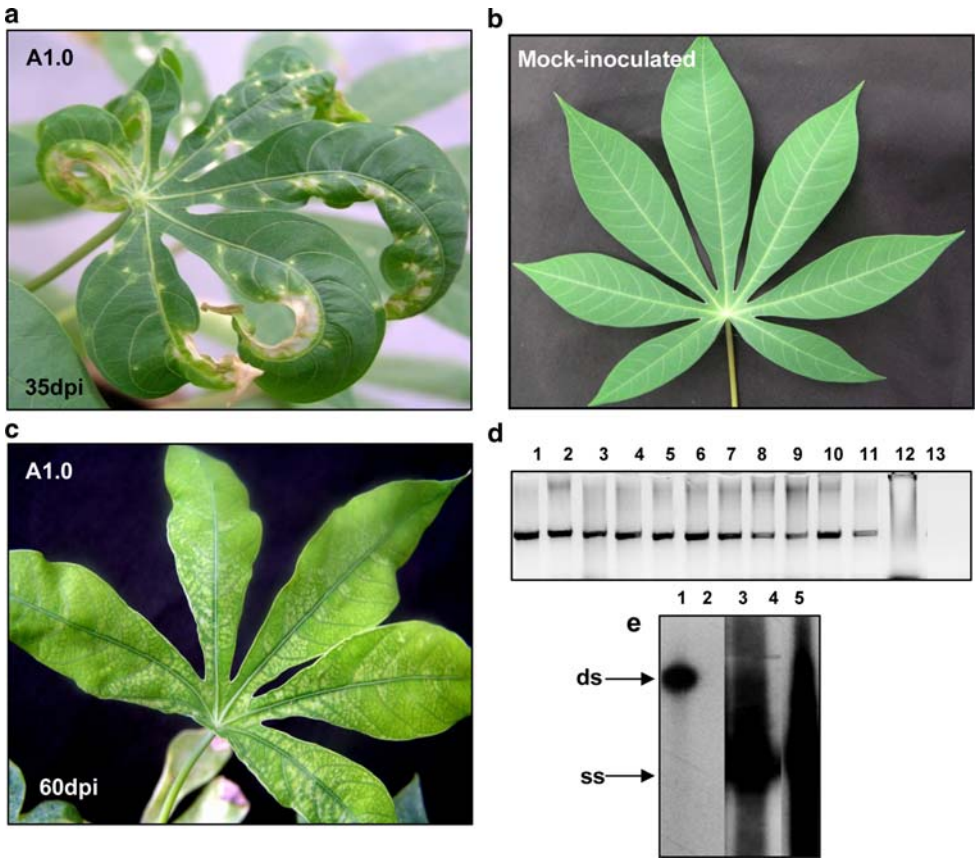

Fig. 2 Infectivity of SLCMV DNAs on cassava. **a** Symptoms on a leaf showing deformity and necrosis, along with the DNA used for inoculation and the period after which the photograph was taken; **b** leaf from a mock-inoculated plant. **c** Mild mosaic on a leaf from a plant inoculated with the DNA shown. **d** PCR amplification products indicating SLCMV DNA-A in leaves showing deformity and necrosis; *lanes* 1–10, inoculated plants at 35 dpi., *lane* 11, naturally infected plant (collected from the field), *lanes* 12 and 13, mock-inoculated plants. **e** Southern hybridization of DNA extracted from cassava leaves; *lanes* 1 and 2, 5 ng cloned DNA-A and DNA-B, respectively, *lane* 3, DNA from a leaf of an inoculated plant exhibiting mosaic symptoms, *lane* 4, DNA from a mock-inoculated plant, *lane* 5, DNA from a CMD-affected plant. The blot was probed with SLCMV DNA-A; the positions of single-stranded (*ss*) and double-stranded (*ds*) DNAs are indicated by *arrows*

indicate the monopartite nature of SLCMV-[Ker20] DNA-A, the second such example after SLCMV-[Col] DNA-A [18], reinforcing the evidence that SLCMV behaves like a monopartite begomovirus and extending such behaviour in three additional hosts, namely *N. tabacum*, Arabidopsis and cassava.

Agroinoculation as a means to introduce cloned geminiviral DNAs to cassava had been reported earlier [2]. In this study, we show that agroinoculation can be used, albeit with low efficiency, to introduce SLCMV DNA to cassava. Thus, agroinoculation, in addition to biolistic inoculation, potentially opens up another whitefly-independent means of screening a large amount of cassava germplasm for resistance against SLCMV. It also provides an opportunity to test the effects of site-directed mutagenesis on the infectivity of the viral DNA and symptom development in the natural host. Interestingly, A1.0 was infectious to cassava, independent of DNA-B, thus illustrating for the first time its monopartite nature in its natural host.

This is also the first report of infection of the model plant Arabidopsis with SLCMV DNA. An interesting feature of the viral DNA extracted from such infected plants was its reduced electrophoretic mobility. This may result from accumulation of multimeric forms of the viral DNA, as has been reported recently in the case of a newly emerged recombinant DNA component of a geminivirus infecting *Sida micrantha* [7]. It has been proposed that such multimeric forms may arise due to the inability of the replication complex to recognize initiation and termination sequences efficiently due to poor adaptation of the virus to the host. Such phenomena might also be occurring between SLCMV and Arabidopsis. The symptoms observed in Arabidopsis resembled those reported earlier for another geminivirus, beet curly top virus [8, 11], suggesting

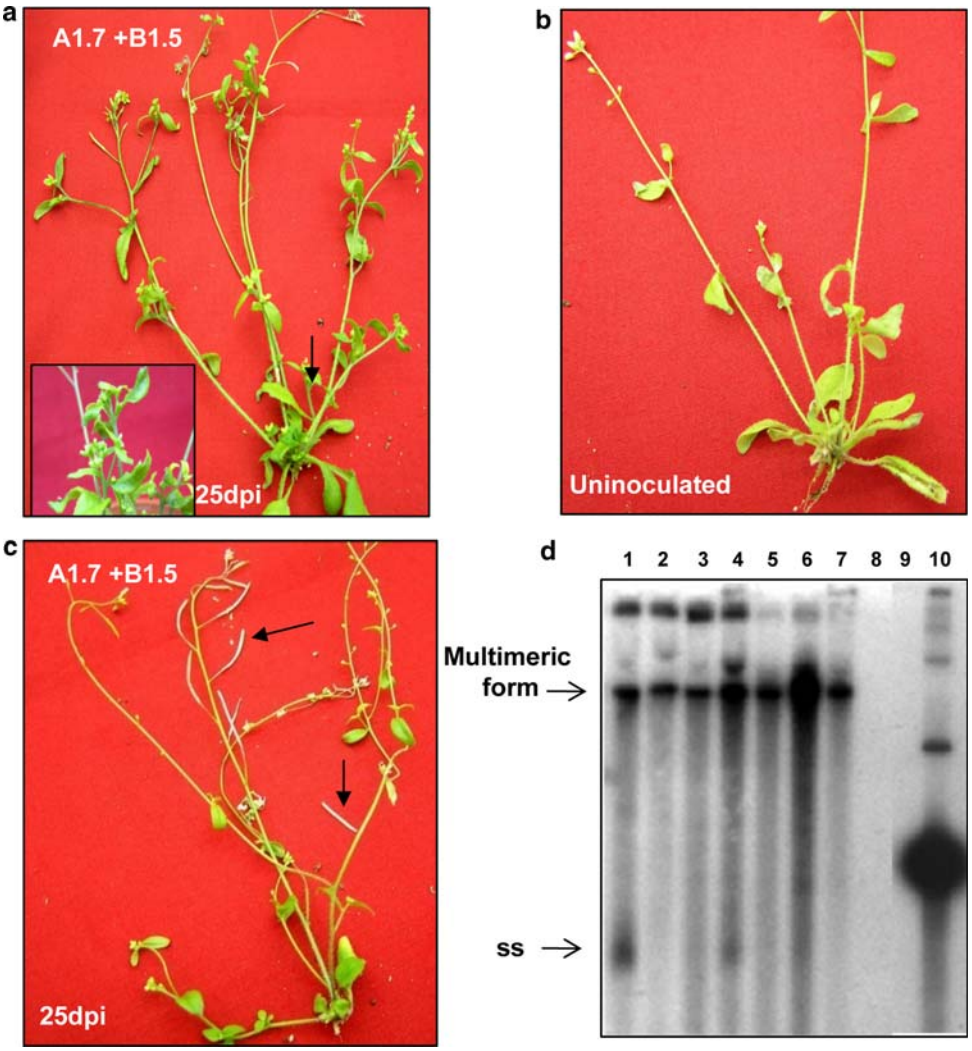

Fig. 3 Infectivity of SLCMV DNAs on Arabidopsis. **a** A plant inoculated with the DNA indicated, showing extensive branching, development of floral buds (inset) and secondary rosette (indicated by an *arrow*), whereas no symptoms are seen in a mock-inoculated plant (**b**). **c** Inoculated plant showing early dehiscence of siliques (indicated by *arrows*). Southern hybridization analysis of inoculated Arabidopsis plants (**d**). *Lanes* 1–7, total genomic DNA from plants inoculated with A1.7 + B1.5, *lanes* 8 and 9, DNA from a mock-inoculated plant, *lane* 10, 5 ng of cloned and linearized DNA-A. The blot was probed with SLCMV DNA-A probe. Positions of multimeric forms and ss-DNA are indicated by *arrows*

possible common interacting pathways between plant factors and closely related viruses. Agroinoculation of Arabidopsis opens up the possibility of using its extensive genomic information and mutant lines available to study interaction with SLCMV.

Acknowledgments B. K. B. is thankful for the award of a Research Fellowship by the Council of Scientific and Industrial Research, New Delhi.

References

1. Ariyo OA, Atri GI, Dixon AGO, Winter S (2006) The use of biolistic inoculation of cassava mosaic begomovirus in screening for resistance to cassava mosaic disease. J Virol Methods 137:143
2. Berrie LC, Rybicki EP, Rey MEC (2001) Complete nucleotide sequence and host range of *South African cassava mosaic virus*: further evidence for recombination amongst begomoviruses. J Gen Virol 82:53–58
3. Briddon RW, Liu S, Pinner MS, Markham PG (1998) Infectivity of African cassava mosaic virus clones to cassava by biolistic inoculation. Arch Virol 143:2487–2492
4. Dutt N, Briddon RW, Dasgupta I (2005) Identification of a second begomovirus, Sri Lankan cassava mosaic virus, causing cassava mosaic disease in India. Arch Virol 150:2101–2108
5. Fauquet C, Fargette D (1990) African cassava mosaic virus: etiology, epidemiology and control. Plant Dis 74:404–411
6. Grimsley N, Hohn B, Hohn T, Walden R (1986) Agroinfection an alternate route for viral infection of plants by using the Ti plasmid. Proc Natl Acad Sci USA 83:3282–3286
7. Jovel J, Preiss W, Jeske J (2007) Characterization of DNA intermediates of an arising geminivirus. Virus Res 130:63–70
8. Lee S, Stenger DC, Bisaro DM, Davis KR (1994) Identification of loci in Arabidopsis that confer resistance to geminivirus infection. Plant J 6(4):525–535
9. Malathi VG, Nair NG, Shanta P (1985) *Cassava mosaic disease.* Technical Bulletin Series 5, Central Tuber Crops Research Institute, Trivandrum, India, pp 18
10. Maule AJ, Hull R, Donson J (1983) The application of spot hybridisation to the detection of DNA and RNA viruses in plant tissues. J Virol Methods 6:215–224
11. Park J, Hwang H, Shim H, Im K, Auh C, Lee S, Davis KR (2004) Altered cell shapes, hyperplasia and secondary growth in Arabidopsis caused by beet curly top geminivirus infection. Mol Cell 17:117–124
12. Patil BL, Rajasubramanium S, Bagchi C, Dasgupta I (2005) Both Indian cassava mosaic virus and Sri Lankan cassava mosaic virus

are found in India and exhibit high variability as assessed by PCR RFLP. Arch Virol 150:389–397
13. Patil BL, Dutt N, Briddon RW, Bull SE, Rothenstein D, Borah BK, Dasgupta I, Stanley J, Jeske H (2007) Deletion and recombination events between the DNA-A and DNA-B components of Indian cassava-infecting geminiviruses generate defective molecules in *Nicotiana benthamiana*. Virus Res 124:59–67
14. Roberts CS, Rajagopal S, Smith LA (1998) A comprehensive set of modular vectors for advanced manipulations and efficient transformation of plants by both *Agrobacterium* and direct DNA uptake methods. CAMBIA Vector Release Manual Version 3.5
15. Rothenstein D, Briddon RW, Haible D, Stanley J, Frischmirth T, Jeske H (2005) Biolistic infection of cassava using cloned components of Indian cassava mosaic virus. Arch Virol 150:1169–1175
16. Rothenstein D, Haible D, Dasgupta I, Dutt N, Patil BL, Jeske H (2006) Biodiversity and recombination of cassava-infecting begomoviruses from southern India. Arch Virol 151:55–69
17. Sambrook J, Russell R (2001) Molecular cloning: a laboratory manual. Cold Spring Harbor Press, Cold Spring Harbor
18. Saunders K, Stanley J (1999) A nanovirus-like DNA component associated with yellow vein disease of *Ageratum conyzoides*: evidence for interfamilial recombination between plant DNA viruses. Virology 264:142–152
19. Saunders K, Salim N, Mali VR, Malathi VG, Briddon R, Markham PG, Stanley J (2002) Characterisation of Sri Lankan cassava mosaic virus and Indian cassava mosaic virus: evidence for acquisition of a DNA B component by a monopartite begomovirus. Virol 293:63–74
20. Stanley J (1983) Infectivity of the cloned geminivirus genome requires sequences from both DNAs. Nature 305:643–645
21. Stanley J, Gay MR (1983) Nucleotide sequence of cassava latent virus DNA. Nature 301:260–262
22. Stenger DC, Revington GM, Stevenson MC, Bisaro DM (1991) Replicational release of geminivirus genomes from tandemly repeated copies, evidence for rolling circle replication of a plant viral DNA. Proc Natl Acad 88:8029–8033