REPLACING THE AC2/AC3 GENES OF ABUTILON MOSAIC VIRUS (ABMV) WITH THOSE OF BEAN DWARF MOSAIC VIRUS ENHANCES ABMV ACCUMULATION, MOVEMENT, AND SYMPTOM SEVERITY IN BEAN

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SUMMARY

Abutilon mosaic virus (AbMV) and Bean dwarf mosaic virus (BDMV) are phylogenetically related, bipartite-genome begomoviruses. AbMV is limited to the plant host phloem but BDMV is not. We have previously provided evidence that the genomic DNA-A component of BDMV contains determinants involved in movement (Levy and Czosnek, 2003). We report here that the DNA-A-encoded genes AC2 and AC3 are involved in virus accumulation and spread. To follow AbMV and BDMV movement in inoculated bean plants, we replaced their coat protein genes (CP) with DNA encoding the green or the red fluorescent proteins (GFP, RFP) to create BDMV-CP:GFP, BDMV-CP:RFP, and AbMV-CP:GFP. Frame-shift mutations in BDMV AC2 and AC3 to produce BDMV-CP:GFP-mC23 resulted in inhibition of BDMV movement when co-inoculated with BDMV DNA-B. The mutation reverted to wild-type and movement was restored when BDMV-CP:GFP-mC23 was co-inoculated with BDMV-CP:RFP and BDMV DNA-B, which strongly suggests that the AC2/AC3 region is important for BDMV movement. Consequently we replaced the AC2/AC3 region of AbMV-CP:GFP with that of BDMV to create AbMV-CP:GFP-C23: BDMV. AbMV-CP: GFP-C23: BDMV inoculated together with AbMV DNA-B moved from cell to cell in the epidermis towards the phloem, and long-distance in the entire plant, even though inocula lacked part of all of BDMV DNA-B. AbMV-CP:GFP with its cognate DNA-B was unable to move. Inoculation of bean with AbMV-CP:GFP-C23:BDMV and BDMV DNA-B resulted in the accumulation of very large amounts of viral DNA, in remarkably fast systemic movement, and in the early appearance of severe symptoms, which in most cases, resulted in an inhibition of germination. These results suggest that interactions between AC2/AC3 of BDMV and other proteins encoded by AbMV DNA-A and DNA-B influence the ability of AbMV to overcome the phloem barrier and move in non-phloem cells. Furthermore, they suggest that proteins encoded by AbMV DNA-A also interact with those encoded by BDMV DNA-B, resulting in an enhancement of systemic spread and of symptom development.

Key words: Abutilon mosaic virus, Bean dwarf mosaic virus, begomovirus, virus movement, AC2 and AC3 genes.

INTRODUCTION

Bean dwarf mosaic virus (BDMV) and Abutilon mosaic virus (AbMV) are bipartite genome, whitefly-transmitted geminiviruses (genus Begomovirus, family Geminiviridae). Their genomes are divided into two circular single-stranded DNA molecules (DNA-A and DNA-B) of about 2,700 nucleotides each (Frischmuth et al., 1990; Hidayat et al., 1993). The function of the geminivirus genes has been the object of many studies (reviewed by Hanley-Bowdoin et al., 1999; Brown, 2001). DNA-A contains four large open reading frames (ORF) while DNA-B contains two ORFs. The DNA-A virion strand contains a single ORF (AV1) that encodes the coat protein (CP). The CP is necessary for transmission by the whitefly vector Bemisia tabaci but not for movement of bipartite begomoviruses in plants. The DNA-A complementary strand contains three ORFs. AC1 codes for a replication associated protein (Rep), AC2 for a transcriptional activator protein (TrAP) and AC3 for a replication enhancer protein (Ren). Both BV1 and BC1 are essential for virus cell-to-cell and systemic movement.

Some bipartite geminiviruses (e.g. AbMV) are limited to the phloem of infected plants while others can invade many tissues (e.g. BDMV). The genetic determinants of cell-to-cell and long-distance movement of the bipartite begomoviruses and their tissue tropism have been studied extensively by using pseudorecombinants between phloem-limited and non-phloem-limited virus-
es, and by swapping homologous genes from viruses belonging to either type (Gillette et al., 1998; Wege et al., 2000). These experiments have shown that movement of some viruses in phloem and non-phloem tissues is dependent on the interaction between genes located on DNA-A and on DNA-B.

Using BDMV and AbMV GFP:CP replacements, we have shown previously that DNA-B of BDMV can overcome the phloem limitation of AbMV (Levy and Czosnek, 2003). In the reciprocal case, the B genomic component of AbMV could not influence the movement of BDMV DNA-A. Hence, we concluded that, in addition to the two gene products BC1 and BV1, the A genomic component of BDMV includes determinants involved in movement of the virus. In this communication, we show that the AC2/AC3 region of BDMV is necessary for virus movement. We also demonstrate that following the replacement of AbMV AC2/AC3 genes by those of BDMV, AbMV is able to move into the phloem and long-distance when co-inoculated with its cognate DNA-B. In this investigation we also showed that accumulation of AbMV, velocity of movement and disease symptoms were greatly exacerbated when AbMV-CP:GFP-C23:AbMV, velocity of movement and disease symptoms were greatly exacerbated when AbMV-CP:GFP-C23:AbMV, velocity of movement and disease symptoms were greatly exacerbated when AbMV-CP:GFP-C23:AbMV, velocity of movement and disease symptoms were greatly exacerbated when AbMV-CP:GFP-C23:AbMV, velocity of movement and disease symptoms were greatly exacerbated when AbMV-CP:GFP-C23:AbMV, velocity of movement and disease symptoms were greatly exacerbated when AbMV-CP:GFP-C23:AbMV, velocity of movement and disease symptoms were greatly exacerbated when AbMV-CP:GFP-C23:AbMV, velocity of movement and disease symptoms were greatly exacerbated when AbMV-CP:GFP-C23:AbMV.

The reaction was stopped by adding 1 µl of 0.5 M EDTA. Subsequently, the two blunt ends were ligated. As a result, the four nucleotides TCGA between nt 1,469 and 1,472 were deleted, leading to a +1 frame shift (Fig. 2). In AC2, the deletion resulted in the translation of the first 6 N-terminal amino acids of AC2 followed by the 124 C-terminal amino acids of AC3. In AC3, the deletions lead to the translation of the first 6 N-terminal amino acids followed by a new peptide of 15 new amino acids terminated by a stop codon (Fig. 2). The mutation was confirmed by sequencing.

Replacement of AbMV AC2/AC3 genes with their BDMV homologues to obtain clone AbMV-CP:GFP-C23:BDMV. BDMV DNA-A was released from its vector pBK-RSV with SalI and circularized following ligation. The BDMV AC2/AC3 region was PCR-amplified using primers BDMV-A1541 and BDMV-A1063. Primer BDMV-A1541 (complementary sense, 5'-CATTTGACTTG CAGTGCGGTGTGCTCT-3') introduced a PstI site at AC2 nt 1,534, 90 bp after the ATG start codon (the restriction site is underlined and the nucleotide changes are in italics), without changing the AC2 ORF. Primer BDMV-A1063 (sense, 5'-GATCTATTTTACGATTGCAG-3') ends 6 nts after the AC3 stop codon, which comprises the PstI site. The 479 bp BDMV AC2/AC3 ampiclon was cloned into the pGEM-T easy vector (Promega Corp., Madison, WI, USA) and released from the vector using PstI and XbaI. AC2/AC3 of AbMV was excised from AbMV-CP:GFP as follows. AbMV-CP:GFP was first digested with XbaI and then partially digested with PstI. The 6,638 bp fragment of the AbMV-CP:GFP cloned in the pBK-RSV (without the AC2/AC3 region) was purified following gel electrophoresis (Bioneer, Daejeon, Korea) and introduced into AbMV-CP:GFP lacking its own AC2/AC3. The AbMV-CP:GFP 6,638 bp fragment was ligated with the PCR-amplified BDMV AC2/AC3 to obtain clone AbMV-CP:GFP-C23:BDMV. The integrity of the clone was verified by sequencing.

Germination of bean seeds and seedling inoculation with viral DNA-coated microprojectile. Three days prior to inoculation, bean seeds (Phaseolus vulgaris cv Vax-216) were placed on sterilized 3 MM Whatman filters wetted with sterile water, and incubated in the dark in a growth chamber at 26-28°C. Before bombardment, a full-length virus genome copy was released from the cloning vector by incubation with either SalI (BDMV-CP:GFP, BDMV-CP:RFP and BDMV-CP:GFP-mC23), BamHI (BDMV DNA-B), PstI (AbMV-CP:GFP or AbMV-CP:GFP-C23:BDMV), or XbaI (AbMV DNA-B). Bean seedlings were bombarded with viral DNA-coated micro-projectiles as described by Sudarshana et al. (1998) and Levy and Czosnek (2003).

PCR amplification of viral DNA. DNA was extracted

MATERIALS AND METHODS

Replacement of the coat protein (CP) gene of AbMV and BDMV by the green or the red fluorescent protein (GFP, RFP) genes to obtain clones AbMV-CP:GFP, BDMV-CP:GFP and BDMV-CP:RFP. Full-length copies of DNA A and DNA B of AbMV from the West Indies (Frischmuth et al., 1990, accession numbers X15983, X15984) and of DNA A and DNA B of BDMV from Colombia (Hidayat et al., 1993, accession numbers M88179, M88180) were cloned in pBK-RSV (Stratagene, La Jolla, CA, USA); in the PstI site (AbMV DNA A), the XbaI site (AbMV DNA B), the SalI site (BDMV DNA A), or the BamHI site (BDMV DNA B). The CP genes of BDMV and AbMV were replaced by the green fluorescence protein (GFP), or by the red fluorescence protein (RFP) genes as previously described (Levy and Czosnek, 2003). A succinct genetic map of these clones is shown in Figure 1.

Mutagenesis of AC2 and AC3 genes of BDMV-CP:GFP to obtain clone BDMV-CP:GFP-mC23. AC2 and AC3 were mutated in a region where they overlap (Fig. 1 and 2). One µg of BDMV-CP:GFP DNA was partially digested with SacI (at nts 1,465-1,470 of BDMV DNA-A). Blunt ends were generated after deleting the 3' overhanging ends using T4 DNA polymerase. For this, one µg of restricted DNA was incubated for 20 min at 11°C with 5 units T4 DNA polymerase, 1 mM of each dNTPs, and 0.1 mg ml⁻¹ BSA in a 50 µl reaction.
Fig. 1. Organization of the AbMV and BDMV DNA-A constructs used in this study. All the constructs were cloned in pBK-RSV. Open reading frames (ORF) of the viral genes (AC1 to AC3, AV1) and of the reporter genes (GFP and RFP) are indicated by arrows. The locations of the first and last nucleotide of each ORF are indicated. CR is the common region. The positions of the relevant restriction sites are shown. Numbers in parenthesis indicate location of the first and last nucleotides of BDMV AC2 and AC3 genes in AbMV-CP:GFP-C23:BDMV.
Replacement of ABMV with BDMV genes

From 50 mg of bean leaves as described by Dellaporta et al. (1983). Fifty ng DNA were subjected to PCR in a tube contained 12 µl of 250 µM dNTPs, 3 µl 10x PCR Buffer, 0.3 µl Taq polymerase, 1.2 µl of 10 pmol µl⁻¹ of each primer and H₂O to a final amount of 30 µl. The cycling protocol was as follows: initial denaturation for 3 min at 95°C, 30 cycles of 1 min at 55°C, 1 min at 72°C and 30 sec at 94°C, and termination by 15 min incubation at 72°C. The PCR products were subjected to electrophoresis in 1% agarose gel. BDMV DNA-B was detected by amplifying a 558 bp fragment using the primer pair BDMV-B2242 (sense, 5'-CCAATTGAGAATCCATATTGAGAATGGCC-3') and BDMV-B225 (complementary sense, 5'-GCAGGTGGCAGGGGACGAGGACGACC-3'). AbMV DNA-B was detected by amplifying a 558 bp fragment using the primer pair AbMV-B218 (complementary sense, 5'-GCACCGGGTACACGCGAGTGCCAGAGG3') and AbMV-B2241 (sense, 5'-CTGAGATCCATTTGCCAGAGATCTGGCCG-3'). BDMV-CP:GFP was detected by amplifying a 500 bp fragment using the primer pair BDMV-A1541 (complementary sense, 5'-CATTGACCTGCAGTGCGGTTGCTC-3'), and GFP687 (sense, 5'-GATCAGTCAGGCGCATGGA-3'). AbMV-CP:GFP and AbMV-CP:GFP-C23:BDMV were detected by amplifying a 610 bp fragment using the primer pair AbMV-A1671 (complementary sense, 5'-GAGGTCTACGCACCAGGGTA-3') and GFP687.

Observation of samples. Tissues were observed with a fluorescent microscope (BH2-RFCA, Olympus) and a laser-scanning (confocal) microscope (LSM-510, Zeiss, Oberkochen, Germany). Photographs were usually taken with a 20 x lens.

RESULTS

Mutagenesis of BDMV AC2 and AC3 genes. AC2 and AC3 of BDMV-CP:GFP were mutagenized by deleting four nucleotides in a region where AC2 and AC3 overlap (Fig. 2). The deletion resulted in the translation of the AC2 52 N-terminal amino acids followed by the AC3 124 C-terminal amino acids. In AC3, the deletion resulted in the translation of the first 6 N-terminal amino acids followed by a new peptide of 15 new amino acids (Fig. 3A). The AC2/AC3 mutant was named BDMV-CP:GFP-mC23.

To investigate if the AC2/AC3 mutant is able to move cell-to-cell and long distance, bean seedlings were co-inoculated 3 days after germination with BDMV-CP:GFP-mC23 and BDMV DNA-B (3 trials of 5 seedlings each) or with AbMV DNA-B (3 trials of 5 seedlings each). Examination of bean hypocotyls with a fluorescent microscope at 3, 5 and 11 days after biolistic inoculation (dpi) showed an increase (not shown) in GFP-associated fluorescence located in single isolated epidermal cells (Fig. 3B).

Reversion of BDMV AC2/AC3 mutant to wild type. To determine if wild type AC2 and AC3 gene products could complement the AC2/AC3 mutations, bean seedlings were inoculated with BDMV-CP:GFP-mC23 and BDMV DNA-B, together with BDMV-CP:RFP. To distinguish between mutant and wild type viruses, a reporter wild type AC2/AC3 virus was generated in which the CP gene was replaced by the RFP gene, generating BDMV-CP:RFP (Fig. 1F). Examination of bean hypocotyls 5 and 11 dpi with a confocal microscope showed green and red fluorescence in clusters of epidermal cells at the region of inoculation (Fig. 4A). Observations at 11 dpi showed green and red fluorescence in the phloem of the upper regions of the stem, approximately 5 cm from the inoculation sites (Fig. 4A). At 20 dpi movement of both viruses was identified in veins of mature trifoliate leaves (Fig. 4C). These results suggested that wild type AC2 and AC3 gene products had complemented the mutated AC2/AC3. However, sequencing the mutated virus using primer BDMV-A1541 revealed that the four nucleotides, TCGA, which have been deleted to produce the mutant (Fig. 2), had been added to the BDMV-CP:GFP-mC23 progeny, resulting in reversion of the mutated AC2/AC3 genes to wild type. Taken together, these results confirmed that the AC2/AC3 region of BDMV is crucial for virus movement.

Replacement of AbMV AC2 and AC3 genes by those of BDMV to produce virus AbMV-CP:GFP-C23:BDMV. To determine if BDMV AC2/AC3 genes are able to induce AbMV movement, the AbMV
Fig. 2. Mutagenesis of the BDMV AC2/AC3 region. The nucleotide and amino acid sequences of the wild type BDMV DNA-A, of the AC2 and AC3 open reading frames, and of the mutant AC2/AC3 in BDMV-CP:GFP-mC23, are shown. The deletion of the four nucleotides AGCT (at position 1469) is shown in green.
AC2/AC3 genes were replaced by those of BDMV, producing the chimeric virus AbMV-CP:GFP-C23:BDMV (Fig. 1 C). Fifteen bean seedlings (five seedlings in three independent trials) were inoculated with AbMV-CP:GFP-C23:BDMV together with AbMV DNA-B. AbMV-CP:GFP inoculated with its cognate DNA-B did not move in bean seedlings, even at 45 dpi (Fig. 5 A), as observed previously (Levy and Czosnek, 2003). Similarly, AbMV-CP:GFP co-inoculated with BDMV DNA-B moved in inoculated plants as did BDMV-CP:GFP when co-inoculated with BDMV DNA-B (Fig. 5 C, D). When bean seedlings were inoculated with AbMV-CP:GFP-C23:BDMV together with AbMV DNA-B, fluorescent clusters of epidermal cells were conspicuous at 11 dpi. Moreover, virus-associated fluorescence was observed in the phloem of the upper part of the stem, approximately 5 cm from the inoculation site (Fig. 5 B), although the number of green foci in the lower and upper parts of the stem was significantly lower than when bean seedlings were inoculated with BDMV DNA-B and either AbMV-CP:GFP or BDMV-CP:GFP (Fig. 5 C, D). Examination of veins of mature trifoliolate leaves did not reveal the presence of virus-associated fluorescence, even at 45 dpi, and the plants remained symptomless. In contrast green fluorescence was observed at 45 dpi in the veins of leaves of plants inoculated with BDMV DNA-B together with either AbMV-CP:GFP or BDMV-CP:GFP (Fig. 5 C, D); these plants exhibited disease symptoms (not shown).

PCR analyses of DNA from mature trifoliolate leaves 11 days after inoculation with AbMV-CP:GFP-C23:BDMV and AbMV DNA-B, revealed that 4 of the 5 plants
examined contained detectable AbMV DNA. In contrast, AbMV DNA was not found in the leaves of the 5 plants inoculated with AbMV-CP:GFP and AbMV DNA-B. Identical results were obtained at 45 dpi (not shown).

Movement and symptoms in bean inoculated with AbMV-CP:GFP-C23:BDMV and BDMV DNA-B. The role of the AC2/AC3 genes in virus movement was further investigated by co-inoculating 30 bean seedlings (5 seedlings in 6 independent trials) with AbMV-CP:GFP-C23:BDMV and BDMV DNA-B. Examination of cross-sections through the hypocotyls of bean seedlings inoculated with AbMV-CP:GFP-C23:BDMV and BDMV DNA-B (Fig. 6 C) showed virus-associated fluorescence as early as 12 h post-inoculation (0.5 dpi).

At that time, not only did large patches of epidermal cells contain virus at the site of inoculation, but the virus had already moved towards the vascular tissue through the cortex. In contrast, fluorescence was not observed 0.5 dpi in the control plants inoculated either with AbMV-CP:GFP and BDMV DNA-B or with BDMV-CP:GFP and BDMV DNA-B (Fig. 6 A, B). By 1 dpi AbMV-CP:GFP-C23:BDMV had moved massively toward the cortex and invaded the vascular tissues (Fig. 6 C). In contrast, plants inoculated with AbMV-CP:GFP and BDMV DNA-B, had small patches of 5 to 10 fluorescent cells in the epidermis of the hypocotyls, at the site of inoculation. AbMV had moved from the epidermis toward the cortex cells, although fluorescence was absent from the vascular cells. In plants inoculated with BDMV-CP:GFP and BDMV DNA-B, sepa-

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**Fig. 4.** Bean seedlings inoculated with the AC2/AC3 mutant BDMV-CP:GFP-mC23 together with BDMV-CP:RFP and BDMV DNA-B. Observation of GFP (I), RFP (II) and both GFP and RFP (III) in **A** epidermis from a lower part of the stem, at the inoculated region, 11 dpi; **B** cross section of the upper part of the stem, approximately 5 cm from the inoculated region, 11 dpi; **C** mature trifoliolate leaf at 20 dpi. Vascular tissues inoculated with GFP or RFP are indicated by green and red arrows. E: epidermis, C: cortex, VC: vascular cylinder. Scale bar = 100 µm.
rate single glowing cells could be seen on the epidermis of the inoculated hypocotyls but no cell-to-cell movement was detectable (Fig. 6 A).

At 6 dpi all the control plants inoculated with AbMV-CP:GFP and BDMV DNA-B, or with BDMV-CP:GFP and BDMV DNA-B, were symptomless. In contrast, plants inoculated with AbMV-CP:GFP-C23:BDMV and BDMV DNA-B showed strong or complete inhibition of growth (Fig. 7 A). Examination with a confocal microscope revealed that in leaves of severely stunted plants, AbMV-associated fluorescence was conspicuous in the veins and in the epidermal cells (Fig. 7 B). Fluorescence was not seen in the leaves of the inoculated control plants. These results indicated that AbMV-CP:GFP-C23:BDMV (inoculated together with BDMV DNA-B) replicated, moved exceptionally quickly from cell to cell and over long distances in the inoculated bean plants, and induced extremely severe symptoms.

Fig. 5. Cell-to-cell and long-distance movement of bean seedlings inoculated with A) AbMV-CP:GFP and AbMV DNA-B, B) AbMV-CP:GFP-C23:BDMV and AbMV DNA-B, C) AbMV-CP:GFP and BDMV DNA-B, D) BDMV-CP:GFP and BDMV DNA-B. Observation of GFP in the epidermis (site of inoculation), in cross-sections of the lower stem (site of inoculation), in cross-sections of the upper stem (approximately 5 cm from the inoculation site) (at 11dpi), and in mature trifoliolate leaves (at 20 dpi). E: epidermis, C: cortex, VC: vascular cylinder, P: phloem, V: veins. Scale bar = 100 µm.
Fig. 6. Movement of AbMV in bean inoculated with AbMV-CP:GFP-C23:BDMV and BDMV DNA-B (C) Control plantlets were inoculated with BDMV-CP:GFP and BDMV DNA-B (A), and with AbMV-CP:GFP and BDMV DNA-B (B). Fluorescent cells and structures have been observed at the site of inoculation at 0.5 and 1 dpi, in cross-sections of the stem and in the epidermis of the treated hypocotyls. E: epidermis, C: cortex, VC: vascular cylinder, P: phloem. Scale bar = 100 µm.
Accumulation of viral DNA in bean seedlings inoculated with AbMV-CP:GFP-C23:BDMV and BDMV DNA-B. PCR analyses of DNA extracted from leaves showed that AbMV-CP:GFP-C23:BDMV was present in all the plants analyzed, whether the chimeric virus was inoculated together with the DNA-B of AbMV or with that of BDMV (data not shown). The amount of viral DNA in the bean plants 11 days after inoculation with AbMV-CP:GFP-C23:BDMV and BDMV DNA-B was compared with that present in the inoculated control plants using semi-quantitative PCR. All DNA samples were isolated from the same primary leaves. All bean plants were of the same age and were raised under the same conditions. The amounts of genomic DNA in each sample were similar.

Samples were analyzed after 10, 13, 16, 19, 22, 25, 28 and 31 cycles of PCR (Fig. 8). In samples from plants inoculated with AbMV-CP:GFP-C23:BDMV and BDMV DNA-B, AbMV DNA could be detected after 19 cycles and BDMV DNA after 10 cycles. By comparison, in samples from plants inoculated with AbMV-CP:GFP-C23:BDMV and AbMV DNA-B, DNA-A was detected.
after 28 cycles and DNA-B after 22 cycles. In samples from plants inoculated with AbMV-CP:GFP and BDMV DNA-B, AbMV DNA could be detected after 22 cycles and BDMV DNA after 16 cycles. In samples from plants inoculated with BDMV-CP:GFP and BDMV DNA-B, DNA-A was detected after 25 cycles and DNA-B after 19 cycles. Viral DNA was not detected in plants infected with AbMV-CP:GFP and AbMV DNA-B. For each trial, the assays were replicated four times and the results were similar. These results showed that plants inoculated with AbMV-CP:GFP-C23:BDMV and BDMV DNA-B contain amounts of viral DNA far greater than the plants inoculated with the other viral combinations. Hence, replacing the AC2/AC3 gene of AbMV by that of BDMV greatly increased AbMV accumulation in inoculated bean, and the effect was more pronounced when AbMV-CP:GFP-C23:BDMV was co-inoculated with the DNA-B of BDMV than with that of AbMV.

DISCUSSION

Bipartite genome begomoviruses have different patterns of systemic spread in infected plants. Some are strictly restricted to the phloem, while others invade many tissue types. The establishment of a successful infection seems to depend on the interaction of plant host cell factors and viral gene products. The role of the A and B genome components and the genes they encode in these processes has been studied by exchanging whole viral DNA components, genes and DNA segments, between phloem- and non-phloem-restricted begomoviruses (Sung and Coutts, 1995; Hou et al., 1998). We have previously shown that the DNA-B of the non-phloem-limited BDMV induced the movement of the phloem-limited AbMV into mesophyll tissues, while the DNA-B of AbMV was unable to confine BDMV to the phloem. Hence, we concluded that determinants located on BDMV DNA-A are needed for virus movement, in association with the BV1 and BC1 gene products of DNA-B (Levy and Czosnek, 2003).

In the current study, we have examined the role of the AC2/AC3 genes located on the DNA-A of AbMV and BDMV in the tissue tropism of these two viruses. Changed behavior following mutagenesis of the AC2/AC3 genes of BDMV confirmed that this genome region is essential for movement of the virus. The frame-shift mutation resulted in a change in the AC2 open reading frame, producing a
Replacement of ABMV with BDMV genes

ABMV movement, it might change the tissue tropism and the movement pattern of AbMV when transferred to this virus. We have not replaced the entire AC2/AC3 region of AbMV by that of BDMV because in AbMV DNA-A the 24 N-terminal amino acids of AC2 overlap with the C-terminus of AC1 (Fig. 2 and 3). As a result, the region that was replaced started 31 amino acids after the beginning of AC2 and ended immediately after the stop codon of AC3 (and of GFP on the viral genome strand). In order to perform the replacement, we introduced a PstI restriction site in the N-terminus of BDMV AC2 using a mutagenesis primer (the PstI site is present at the same location in the homologous region of AbMV). The PstI restriction site did not change the BDMV AC2 reading frame and the translated amino acids; its location was identical to that of AbMV AC2 (95 bp from the start codon). Therefore, we assumed that BDMV AC2/AC3 region had the potential to function in AbMV as it does in BDMV.

Replacement of AbMV AC2/AC3 genes by their BDMV homologues had dramatic effects on the biology of the virus in inoculated bean plants. Following co-inoculation of AbMV-CP:GFP-C23:BDMV with AbMV DNA-B, AbMV was able to move slowly from cell to cell, from the inoculated epidermal cells into the phloem and then long-distance, which AbMV (wild-type or AbMV-CP:GFP) cannot do. These effects were even more spectacular when AbMV-CP:GFP-C23:BDMV was inoculated with the DNA-B of BDMV. Movement of AbMV-CP:GFP-C23:BDMV was much faster when inoculated with the DNA-B of BDMV than BDMV-CP:GFP inoculated with its cognate DNA-B, or AbMV-CP:GFP co-inoculated with BDMV DNA-B. A few hours after inoculation, AbMV-CP:GFP-C23:BDMV had invaded the phloem and had moved systemically in the plant. One week after inoculation, the viral DNA had accumulated in very large amounts and the inoculated plants showed extremely severe disease symptoms.

These results can best be explained by the current model of bipartite genome begomovirus replication and movement, based on interactions between the genes AC2/AC3 and AC1 located on the DNA-A component, and the genes encoded by DNA-B. AC2 encodes a transcriptional activator protein that activates the expression of the CP and of BV1, which encodes a nuclear shuttle protein. Since in our experiments the CP was replaced by GFP, the interaction between AC2 and CP has no role in replication or movement of the virus. AC2 is known also to interact with cellular kinases, part of the mechanism leading to suppression of the plant defense responses (Hao et al., 2003; Wang et al., 2003). This gene is also known to be a suppressor of gene silencing (Voinnet et al., 1999).Suppressions of defense response and gene silencing could affect viral movement. This feature of AC3, and the interaction between AC2 of AbMV-CP:GFP-C23:BDMV and BV1 of the AbMV DNA-
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