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Bhendi Yellow Vein Mosaic Disease in India Is Caused by Association of a DNA β Satellite with a Begomovirus

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Yellow vein mosaic disease is the major limitation in the production of bhendi or okra (*Abelmoschus esculentus*), an important vegetable crop of India. This disease is caused by a complex consisting of the monopartite begomovirus *Bhendi yellow vein mosaic virus* (BYVMV, family: *Geminiviridae*) and a small satellite DNA β component. BYVMV can systemically infect bhendi upon agroinoculation but produces only mild leaf curling in this host. DNA β induces typical symptoms of bhendi yellow vein mosaic disease (BYVMD) when co-agroinoculated with the begomovirus to bhendi. The DNA β component associated with BYVMD has a number of features in common with those reported for ageratum yellow vein disease and cotton leaf curl disease. BYVMV represents a new member of the emerging group of monopartite begomoviruses requiring a satellite component for symptom induction.

Key Words: BYVMD; DNA β; begomovirus; satellite DNA; aetiology; agroinoculation

INTRODUCTION

Bhendi yellow vein mosaic virus (BYVMV) belongs to the genus *Begomovirus* in the family *Geminiviridae*. Geminiviruses make up a large, diverse family of plant viruses that infect a broad variety of food and fiber crops and cause significant losses worldwide. The majority of begomoviruses have a genome comprising two similar-sized DNA components (DNA A and DNA B). The DNA A component encodes a replication-associated protein (Rep) that is essential for viral DNA replication, a replication enhancer protein (REn), the coat protein (CP) and a transcription activator protein (TrAP) that controls late gene expression. The DNA B component encodes a nuclear shuttle protein (NSP) and a movement protein (MP), both of which are essential for systemic infection of plants (Hanley-Bowdoin *et al.,* 1999; Gafni and Epel, 2002). In contrast, some begomoviruses have only a single genomic component which resembles DNA A, such as isolates of *Tomato yellow leaf curl virus* (TYLCV), *Tomato leaf curl virus* (TLCV), *Ageratum yellow vein virus* (AYVV) and *Cotton leaf curl virus* (CLCuV) (Kheyr-Pour *et al.,* 1991; Navot *et al.,* 1991; Dry *et al.,* 1993; Tan *et al.,* 1995; Briddon *et al.,* 2000).

BYVMV (Brunt *et al.,* 1996) was reported from Bombay in India (Kulkarni, 1924). This is the earliest report of this virus, implying that BYVMV might have originated in India. It has been shown to be a geminivirus based on its morphology and its serological relationship with *African cassava mosaic virus* (Harrison *et al.,* 1991). Bhendi plants infected by BYVMV show persistent symptoms of vein clearing followed by yellowing. Leaves and fruits are reduced in size and there is a significant decrease in the production of the vegetable. Up to 96% loss in yield has been reported (Pun and Doraiswamy, 1999).

Based on the sequences of the Pakistan isolates of *Okra yellow vein mosaic virus* (OYVMV) and CLCuV, Zhou *et al.* (1998) proposed that OYVMV had recombined with another unidentified geminivirus in okra to produce a virus capable of infecting cotton to epidemic proportions in Pakistan. Only DNA A components have been found for nine virus isolates from cotton and okra including OYVMV from Pakistan (Zhou *et al.,* 1998).

Diseased *Ageratum conyzoides* plants contain a satellite DNA, referred to as DNA β. AYVV and DNA β together form a disease complex that is responsible for the yellow vein phenotype (Saunders *et al.,* 2000). Apart from the sequence TAATATTAC, common to all geminiviruses and containing the initiation site of rolling circle replication, DNA β shows negligible sequence homology either to AYVV DNA or to DNA B components associated with other begomoviruses. DNA β depends on AYVV for replication, and is encapsidated by AYVV-encoded coat protein. Systemic infection of *A. conyzoides* with AYVV alone is asymptomatic and viral DNA accumulation is reduced to 5% or less of that in the presence of DNA β. Similarly, cotton leaf curl disease (CLCuD) in Pakistan is caused by CLCuV and an associated DNA β component (Briddon *et al.,* 2001). More recently, Mansoor *et al.* (2001) reported the association of a DNA β component with

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okra leaf curl disease in Pakistan. Plants affected by ageratum yellow vein disease (AYVD) and CLCuD also contain small autonomously-replicating nanovirus-like components, called DNA 1 and DNA 2, in addition to DNA β and begomovirus components (Mansoor *et al.,* 1999; Saunders and Stanley, 1999; Saunders *et al.,* 2002).

PCR-amplification using begomovirus-specific primers showed the presence of a begomovirus component equivalent to DNA A in diseased bhendi plants (Jose and Usha, 2000). Rigorous attempts to amplify a second genomic component (DNA B) were not successful, suggesting that BYVMV is a monopartite begomovirus. However, although infectious, full-length clones of BYVMV were unable to induce symptoms typical of BYVMD in bhendi. In the present work, we have isolated a DNA β component from BYVMV-infected bhendi tissue, which, together with BYVMV, causes typical yellow vein disease symptoms in bhendi. BYVMD is only the third disease to be shown to be caused by the association of a begomovirus and a DNA β satellite, resembling complexes associated with AYVD (Saunders *et al.,* 2000) and CLCuD (Briddon *et al.,* 2001).

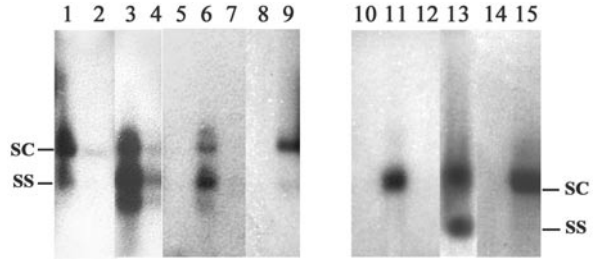

FIG. 1. Detection of BYVMV DNA and DNA β in bhendi plants. Samples (10 μg) of nucleic acids were extracted from individual plants infected either by agroinoculation with BYVMV DNA (Lanes 2 & 4), DNA β (Lanes 7 & 10) or BYVMV DNA and DNA β together (Lanes 1, 3 & 11), or by whitefly-transmission of the progeny acquired from symptomatic plants agroinoculated with BYVMV DNA and DNA β (Lanes 6 & 13). Additional samples are from plants mock-agroinoculated with pBin19 (Lanes 5 & 12), healthy plants (Lanes 8 & 14) and naturally-infected plants from the field (Lanes 9 & 15). The blots were probed either for BYVMV DNA (Lanes 1–9) or for DNA β (Lanes 10–15). The positions of single-stranded (ss) and supercoiled (sc) DNAs are indicated.

RESULTS

Cloning and sequencing of BYVMV DNA

BYVMV DNA was initially amplified using begomovirus DNA A-specific primers, and the 2.7 kbp amplified product was cloned (pBY1) and sequenced. When the sequence was compared with the Pakistan isolate of BYVMV (Acc. No. AJ002453), it was found to contain a deletion in the AV1 open reading frame (ORF) where the reverse primer originally annealed. Therefore, DNA-specific abutting primers were designed to a second region of the Indian isolate and the full-length DNA was amplified and cloned. Four clones (pBY2–pBY5) differing in their restriction patterns were produced. The sequence of pBY5 was derived (Acc. No. AF241479) as it was most similar to pBY1. Coat protein gene sequences of the other clones were derived (Acc. Nos. AJ278860, AJ278861 and AJ278862).

Infectivity and symptoms produced by cloned BYVMV DNA

The infectivity of the partial tandem repeat construct of BYVMV DNA (pBinAPTR7) in bhendi plants was assessed by agroinoculation. The clone was systemically infectious as judged by Southern blot analysis (Fig. 1) but bhendi plants did not show the typical yellow vein mosaic symptoms of the disease. However, curling of the edges of the leaves was observed (Fig. 2).

Attempts to clone a DNA B component

Most clones obtained which showed positive hybridization to the BYVMV intergenic region probe were either full-length or partial clones of the BYVMV DNA A homolog as revealed by their sequence. There were no DNA B clones. PCR reactions using different combinations of primers based on DNA A (abutting primers located in the BYVMV intergenic region) and DNA B (degenerate abutting primers and a forward primer situated in the conserved sequence of the movement protein gene) did not yield a DNA B fragment. This is consistent with the absence of a DNA B component associated with BYVMD.

Cloning and characterization of a DNA β component

An approximately 1.35 kbp DNA β fragment was amplified from diseased bhendi plants using non-overlapping abutting primers (Beta1.F and Beta1.R) located in the highly conserved region found in all DNA β sequences and was cloned into pGEM-T (pBeta1). Association of the cloned DNA with BYVMD was confirmed by Southern blot analysis of DNA extracted from healthy and infected bhendi plants (Fig. 1). BYVMD DNA β-specific primers (Beta2.F and Beta2.R), based on the sequence of pBeta1, were used to amplify a 0.6 kbp fragment. The PCR product, which included the highly conserved region of DNA β, was cloned into pXcmkn12 to produce pBeta2. The sequence of pBeta2 (Acc. No. AJ308425) was identical to that of pBeta1, thereby confirming that no changes had been introduced into the region where the abutting primers annealed.

Analysis of BYVMV DNA A and DNA β in agroinoculated plants

Plants agroinoculated with the partial tandem repeat construct of DNA β (pBinbetaPTR4) were asymptomatic (Fig. 2). To investigate the role of DNA β in BYVMD, bhendi seedlings were agroinoculated with pBinAPTR7 and pBinBetaPTR4. Out of 125 plants agroinoculated in

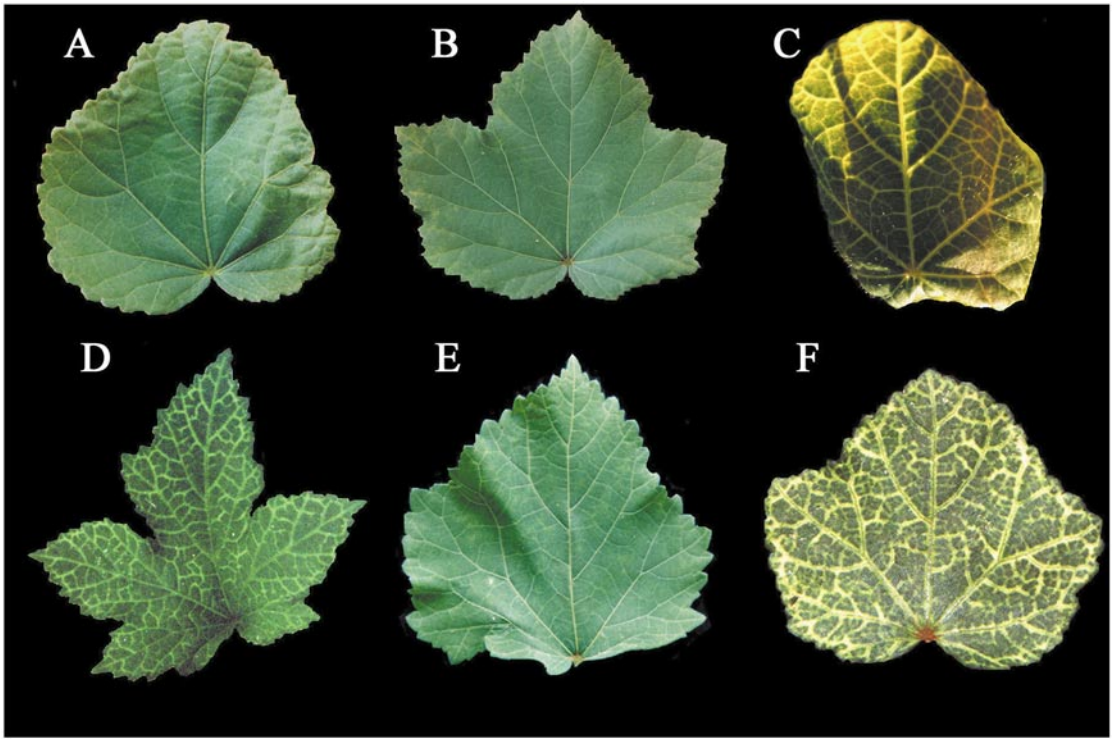

FIG. 2. Infectivity of BYVMV and DNA β in bhendi plants. Leaves are from plants either agroinoculated with BYVMV DNA (A), DNA β (B) or BYVMV DNA and DNA β together (C), mock-agroinoculated with pBin19 (E) or infected using viruliferous whiteflies (D) that had acquired virus from a symptomatic agroinoculated plant (as in panel C). Leaves were photographed either 25 days after agroinoculation or 15 days after whitefly transmission. A naturally infected leaf from the field exhibiting typical BYVMD symptoms (F) is also shown.

three different experiments, 16 plants developed typical yellow vein symptoms of BYVMD by 25 days post-inoculation (p.i.) (Fig. 2). The presence of DNA A and DNA β in systemically infected tissues was confirmed by Southern blot hybridization (Fig. 1).

Southern blot analysis of the plants agroinoculated with pBinAPTR7 alone showed the presence of BYVMV DNA in the newly emerging leaves 45 days p.i. (Fig. 1), demonstrating that replication and systemic movement of the viral DNA had occurred in the absence of DNA β. However, BYVMV DNA accumulated to significantly lower levels in the absence of DNA β.

Whitefly transmission of the progeny virus

Individual symptomatic plants that had been infected by agroinoculation with pBinAPTR7 and pBinBetaPTR4 were used in three separate experiments to transmit the disease to healthy bhendi plants using *Bemisia tabaci.* Typical yellow vein symptoms developed in the test plants after 15 days (Fig. 2). The presence of both DNA A and DNA β in these plants was confirmed by Southern blot hybridization (Fig. 1).

Features of BYVMV and DNA β

BYVMV has a genome organization that is typical of other Old World begomoviruses. The phylogenetic relationship of BYVMV, determined using CLUSTALW, revealed it to be most closely related to the Pakistan isolate of BYVMV (Acc. No. AJ002453; 92.5% identity) and less so to OYVMV (Acc. No. AJ002451; 89% identity) and a Pakistan isolate of CLCuV (Acc. No. AJ002455; 82% identity). The iterated BYVMV sequence TGAGACCC, occurring at positions 2599–2606 and 2657–2664 in the intergenic region upstream of the stem-loop structure, is predicted to be the putative Rep binding site.

The satellite DNA associated with infected bhendi is organized similarly to those associated with AYVD and CLCuD (data not shown), and will be referred to as BYVMD DNA β, in line with the nomenclature adopted for these satellite DNAs (Saunders *et al.,* 2000; Briddon *et al.,* 2001). Using the system adopted for geminivirus components, nucleotide numbering for BYVMD DNA β starts from the 3′ A residue located in the conserved nonanucleotide TAATATTAC. This motif, which DNA β shares with geminiviruses, forms the loop of a predicted stem-loop structure that contains the nick site for initiation of virion-sense DNA replication (Laufs *et al.,* 1995; Stanley, 1995). BYVMD DNA β contains an A-rich region between nucleotides 775 and 975 (60% A residues) and an ORF (βC1) located in the complementary-sense strand with a coding capacity of 16.5 kDa. This ORF was predicted to be functional by the program TESTCODE

FIG. 3. Multiple alignment of DNA β sequences. The shaded regions indicate conserved nucleotides and iterated sequences are boxed. The origin and accession numbers of the DNA β sequences are as follows: BYVMD (AJ308425), CLCuD from India (CLC-In, AY083590) and Pakistan (CLC-Pk1, AJ292769; CLC-Pk2, AJ298903), and TLCV satellite DNA (TLCVsat, U74627).

(Devereux *et al.,* 1984), as previously suggested for AYVD DNA β (Saunders *et al.,* 2000). A putative promoter (nucleotides 853 to 603), transcription factor binding site (TATA box at nucleotide 627) and polyadenylation signal (beginning at nucleotide 189) may contribute to βC1 expression.

Comparison of the sequence of BYVMD DNA β with previously established DNA β sequences shows relatively low sequence identity (AYVD DNA β, 53.7%; CLCuD DNA β (Pakistan), 60.2%; CLCuD DNA β (India), 58.5%; TLCV DNA satellite, 47.9%). However, multiple alignment of the above sequences (Fig. 3) shows a highly conserved stretch of approximately 86 nucleotides upstream of the predicted stem-loop structure. The putative replicase-binding motif present in BYVMV DNA is not found in DNA β. However, the sequence GCTACGC occurs twice in the conserved region of all DNA β sequences, corresponding to nucleotides 1300–1306 and 1317–1323 of BYVMD DNA β.

DISCUSSION

Agroinoculation studies with BYVMV DNA alone demonstrated that although the begomovirus component can autonomously replicate and move systemically in bhendi plants, it is unable to induce the symptoms typical of BYVMD. This prompted us to look for an additional factor, such as a begomovirus DNA B component or a DNA β satellite component similar to those isolated from plants affected with AYVD and CLCuD (Saunders *et al.,* 2000; Briddon *et al.,* 2001). An extensive search failed to reveal a DNA B component, as was also the case for AYVV and CLCuV (Tan *et al.,* 1995; Briddon and Markham, 2000). However, a DNA β component was successfully amplified using a pair of universal primers based on a highly conserved region found in all DNA β sequences examined to date.

The association of BYVMV DNA and DNA β with diseased plants was evident when DNA extracts from naturally infected plants were analyzed by Southern blot hybridization using probes specific for these components. Agroinoculation of bhendi plants using these components confirmed that typical BYVMD symptoms could be produced only when both were present. The requirement for a begomovirus and its associated satellite component to produce typical disease symptoms has previously been demonstrated for AYVD and CLCuD (Saunders *et al.,* 2000; Briddon *et al.,* 2001). In addition, it has been shown that the progeny of the cloned BYVMV DNA and DNA β can be whitefly-transmitted to bhendi plants and that the plants subsequently develop typical BYVMD symptoms. Previously, this has been demonstrated only for AYVD using the weed species *A. conyzoides*. The ability to reproduce the BYVMD phenotype using cloned DNA components fulfills Koch's postulates for this disease.

The ability of BYVMV to accumulate, albeit to a low level, and to systemically move in bhendi plants in the absence of DNA β, and its amplification to normal levels in the presence of DNA β (Fig. 2), resembles the behavior of AYVV and CLCuV (Saunders *et al.,* 2000; Briddon *et al.,* 2001). The DNA A components of some bipartite begomoviruses, for example *African cassava mosaic virus* (Klinkenberg and Stanley, 1990), *Abutilon mosaic virus* (Evans and Jeske, 1993), and *Tomato golden mosaic virus* (Briddon and Markham, 2001), are also capable of systemic movement in the absence of a DNA B component under certain conditions. Thus, both DNA β and DNA B serve to augment the accumulation of DNA A in plants. DNA B encodes proteins required for virus move-

ment (Gafni and Epel, 2002), and it was suggested that DNA β–mediated amplification of the begomovirus DNA may involve either systemic movement, conditioning of cells to make them permissive for replication, or suppression of a host defense mechanism (Saunders, *et al.,* 2000). Subsequently, it was concluded that symptom development primarily resulted from the host response to a DNA β gene product rather than DNA β-mediated amplification of a begomovirus-encoded symptom determinant (Saunders *et al.,* 2001). It has been demonstrated that DNA β depends on the begomovirus for its replication and encapsidation (Saunders *et al.,* 2000). Encapsidation of BYVMD DNA β by BYVMV coat protein is implied from the ability to transmit both components using the whitefly vector.

On the basis of current species demarcation criteria (Rybicki *et al.,* 2000), the begomovirus isolated and characterized during the course of this work is considered to be an Indian isolate of BYVMV as it is most closely related to this virus and shows a nucleotide sequence identity of 92.5%. There has been no report of a DNA β component associated with either BYVMV or OYVMV from Pakistan although our findings suggest that they may be involved in similar disease complexes.

BYVMD DNA β has several characteristic features in common with other satellite homologs, namely a conserved nonanucleotide situated in the stem-loop region, a highly conserved region of 86 nucleotides situated upstream of the stem-loop, a conserved βC1 ORF, and an extensive A-rich region. DNA β is structurally distinct from begomovirus components, yet in some respects may be functionally equivalent to DNA B. DNA β has a number of features in common with DNA B, for instance *trans*-replication and encapsidation by begomovirus-encoded proteins and a symptom determinant located in the complementary strand (J. Stanley, personal communication), possibly reflecting the convergent evolution of these components (Saunders, *et al.,* 2001). DNA B components of bipartite begomoviruses share a common region with their cognate DNA A components that contain virion-sense and complementary-sense promoters as well as iterons that have been implicated in Rep binding (Arguello-Astorga *et al.,* 1994). However, such motifs are not conserved between DNA A and DNA β, implying that these components are probably evolutionarily unrelated. Unlike the DNA 1 and DNA 2 components associated with AYVD and CLCuD that are clearly related to nanovirus components (Mansoor *et al.,* 1999; Saunders and Stanley, 1999; Saunders *et al.,* 2000), DNA β does not show significant homologies to any other known sequence, including geminivirus and nanovirus components. Saunders *et al.* (2000) suggested that DNA A and DNA β may have co-existed before the emergence of bipartite begomoviruses and that DNA B usurped DNA β. DNA β has remained undetected until quite recently (Saunders *et al.,* 2000). With the identification of a highly conserved sequence within DNA β homologs, the use of universal primers is enabling the detection of an increasing number of DNA β components in association with distinct monopartite begomoviruses (unpublished results). This raises the question of whether begomoviruses that are reliant on a DNA β component for their maintenance in the field should be considered taxonomically distinct from other monopartite and bipartite viruses in the family *Geminiviridae*.

MATERIALS AND METHODS

Source and maintenance of virus isolate

Diseased bhendi plants were collected from Madurai District in the Tamil Nadu state of India, and maintained in a glasshouse.

Cloning of BYVMV DNA

Total nucleic acids were extracted from diseased bhendi plants as described (Jose and Usha, 2000). Viral DNA was PCR-amplified using virion-sense (CAV1.F) and complementary-sense (CAV1.R) primers (Table 1) based on the conserved sequence of the begomovirus coat protein gene. Initially a 2.7 kbp fragment was amplified and cloned into pGEM-T (Promega) to produce clone pBY1. A pair of BYVMV-specific abutting primers was designed based on the pBY1 sequence (BYV1.F and BYCP2.R; Table 1). Full-length copies of the viral DNA were amplified using these primers and cloned into pGEM-T to produce clones pBY2–pBY5. Clones were analyzed by restriction digestion, and nucleotide sequences of both strands of the cloned DNA were determined using automated DNA sequencing (Microsynth, Switzerland).

Construction of a partial tandem repeat of BYVMV DNA

Full-length BYVMV DNA was re-amplified from clone pBY5 using a pair of overlapping primers (BYVBCP2.R and BYV1.F; Table 1) containing a *Vsp*I site, and was cloned into pGEM-T to produce pBY6. *Bam*HI (1306)–*Vsp*I (1052) and *Eco*RI (675)–*Vsp*I (1052) fragments were released from this clone and both fragments were simultaneously cloned into the *Eco*RI–*Bam*HI sites of pOK12 (Vieira and Messing, 1991) via a three-way ligation. The *Bgl*II (2451)–*Eco*RI (675) fragment from pBY5 was inserted into this clone (pAPTR1) to produce pAPTR2, from which the *Eco*RV (300)–*Bam*HI (1306) fragment was released and cloned into pOK12 to produce pAPTR3. The *Hin*dIII (60)–*Bam*HI (1306) fragment from pBY5 was cloned into pAPTR3 to produce pAPTR4. The *Sal*I–*Eco*RV (300) fragment was released from pBY5 and was cloned into pBSIIKS+ to produce pAPTR5. The *Eco*RV–*Sac*I fragment was released from pAPTR4 and cloned into pAPTR5 to produce pAPTR6. The *Sac*I–*Sal*I fragment was

TABLE 1

Primers Used for PCR

Primer[a]	Sequence[b]	Location
CAV1.F	AGCTGGAAAATATGAAAATC	Conserved region of AV1
CAV1.R	TCCTGCTGGTTATACACAAC	Conserved region of AV1
MPGEM1.F	KBCTRTATTCTAYGACGMMGTGG	Conserved region of BC1
MPGEM2.R	ANCARGTNCCVATHAACGC	Conserved region of BC1
BYV1.F	AATTAATAAAGTTTGAATTTTATATC	BYVMV DNA 1051–1075
BYCP2.R	TCAATTCGTTACAGAGTC	BYVMV DNA 1033–1050
BYCRH.F	AAGCTTAGATAACGCTCCTTC	BYVMV DNA 61–81
BYCRH.R	AAGCTTTGAGTCTGCATCG	BYVMV DNA 48–66
BYVCP2.R	TTATTAATTTCAATTCGTTACAGAGTC	BYVMV DNA 1033–1058
BDNA.F	GCRTTDAHNGGNACYTGNTG	Conserved region of BC1
BYCR.R	TACTGGGGAGAGTTGTAAG	BYVMV DNA 2618–2636
BETA1.F	AGCCTTAGCTACGCCGGAGC	BYVMD DNA β1310–1329
BETA1.R	GCTGCGTAGCGTAGAGGTTT	BYVMD DNA β1290–1309
BETA2.F	TGAGTGGGTAAATTAGACAG	BYVMD DNA β1042–1061
BETA2.R	TGCAGATCAGTTCAACAAG	BYVMD DNA β434–451

[a] F: viral sense; R: complementary sense.
[b] Restriction sites are underlined.

released from pAPTR6 and cloned into pBin19 binary vector to produce a 1.9-mer partial tandem repeat of the viral DNA (pBinAPTR7). The numbers in parentheses indicate the positions of restriction sites in the viral genome. Sites from vectors are not numbered.

Attempts to detect a BYVMV DNA B component

In an attempt to isolate a DNA B component associated with BYVMD, DNA extracted from infected bhendi plants using citrate buffer (pH 6), followed by alkali lysis enrichment of the replicative form of the viral DNA, was digested with a number of restriction enzymes and the fragments were cloned. Clones ranging in size from 200 bp to 2.7 kbp were selected and analyzed by Southern blot hybridization using either a full-length BYVMV DNA probe or probes specific for AV1 and AC1 ORFs or the intergenic region. Clones that hybridized to the intergenic region probe were sequenced.

In an alternative PCR approach, virion-sense primer BYCRH.F and complementary-sense primer BYCRH.R (Table 1) were designed on the nucleotide sequence of the BYVMV DNA intergenic region. Another set of degenerate abutting primers (MPGEM1.F and MPGEM2.R) and a forward primer (BDNA.F) (Table 1) were designed on conserved sequences in the DNA B movement protein gene. Different combinations of the above primers were used in an attempt to amplify DNA B.

Cloning of DNA β

Based on the conserved region of DNA β components associated with AYVV and *Honeysuckle yellow vein mosaic virus* (J. Stanley, personal communication), a pair of non-overlapping abutting primers (Beta1.F and Beta1.R; Table 1) was designed and used to amplify a 1.4 kbp fragment which was cloned into pGEM-T to produce pBeta1.

Construction of a partial tandem repeat of DNA β

A pair of BYVMD DNA β-specific primers (Beta2.F and Beta2.R; Table 1), flanking the above-mentioned conserved region, was used to amplify a 0.6 kbp fragment which was cloned into pXcmkn12 (Cha *et al.,* 1993) to produce pBeta2. The *Kpn*I (1109)–*Eco*RV (236) fragment was released from pBeta2 and was cloned into pBSIIKS+ to produce pBetaPTR1. The *Pst*I–*Kpn*I (1109) fragment was released from pBeta1 and was cloned into pXcmkn12. The *Kpn*I (1109)–*Eco*RI fragment from pBetaPTR1 was inserted into this clone (pBetaPTR2), to produce pBetaPTR3, from which the *Hin*dIII–*Eco*RI fragment was excised and cloned into pBin19 binary vector to produce a 1.2-mer partial tandem repeat of DNA β (pBinBetaPTR4).

Agroinoculation of plants

pBinAPTR7 and pBinBetaPTR4 were mobilized into *Agrobacterium tumefaciens* LBA4404 by triparental mating (Ditta *et al.,* 1980) and the transconjugants were selected on kanamycin (50 μg/ml) and rifampicin (10 μg/ml). The integrity of the cloned DNA in *A. tumefaciens* was verified by Southern blot analysis. Surface-sterilized bhendi seeds were germinated by soaking for 48 h in sterile water on cotton. Agroinoculation was carried out with pBinAPTR7 and pBinBetaPTR4, either alone or mixed together as described (Mandal *et al.,* 1997).

Insect transmission

Non-viruliferous *Bemisia tabaci* were maintained on *Solanum nigrum* plants in an insect-proof cage. Symp-

tomatic bhendi plants infected by agroinoculation were introduced into the cage after one month. Healthy plants (thirty days old) were introduced the following day.

Viral DNA detection

Nucleic acids were isolated from young leaves of bhendi plants by a modified CTAB method (Porebski *et al.,* 1997). DNA was separated on a 1% agarose gel in TAE buffer and was blotted onto nylon membrane (Bio-Bond, Sigma) following alkali denaturation and neutralization (Southern, 1975), and hybridized to randomly-labeled probes (Feinberg and Vogelstein, 1983).

Sequence analysis of BYVMV DNA and DNA β

The sequences of full-length clones of BYVMV and DNA β were analyzed using version 9 of the program library of the University of Wisconsin Genetics Computer Group (Devereux *et al.,* 1984). Homologies with other components were investigated using BLAST searches (Altschul *et al.,* 1997) and a phylogenetic tree was constructed using CLUSTALW. The presence of promoter and transcriptional factor binding sites in DNA β sequences were predicted using version 1.7 of PROSCAN and SIGNAL scan programs (Prestridge, 1991).

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