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A begomovirus associated with ageratum yellow vein disease in Indonesia: evidence for natural recombination between tomato leaf curl Java virus and Ageratum yellow vein virus-[Java]

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Summary

A begomovirus (2747 nucleotides) and a satellite DNAβ component (1360 nucleotides) have been isolated from *Ageratum conyzoides* L. plants with yellow vein symptoms growing in Java, Indonesia. The begomovirus is most closely related to Tomato leaf curl Java virus (ToLCJV) (91 and 98% in the total nucleotide and coat protein amino acid sequences, respectively), although the products of ORFs C1 and C4 are more closely related to those of Ageratum yellow vein virus-[Java] (91 and 95% identity, respectively). For this reason, the begomovirus it is considered to be a strain of ToLCJV and is referred to as ToLCJV-Ageratum. The virus probably derives from a recombination event in which nucleotides 2389–2692 of ToLCJV have been replaced with the corresponding region of the AYVV-[Java] genome, which includes the 5′ part of the intergenic region and the C1 and C4 ORFs. Infection of *A. conyzoides* with ToLCJV-Ageratum alone produced no symptoms, but co-infection with DNAβ induced yellow vein symptoms. Symptoms induced in *Nicotiana benthamiana* by ToLCJV-Ageratum, ToLCJV and AYVV-[Java] are consistent with the exchange of pathogenicity determinant ORF C4 during recombination.

Introduction

Geminiviruses have circular single-stranded DNA genomes with one or two components that are encapsidated in geminate particles. Geminiviruses are currently divided into four genera on the basis of their genome organization and biological properties [15]. Most of the geminiviruses are transmitted by whiteflies and belong to the genus *Begomovirus*. These species have great economic importance and enormous diversity resulting from their widespread geographic distribution and host adaptation [42]. Begomoviruses generally have bipartite genomes (designated as DNA-A and DNA-B) and infect

Nucleotide sequence data reported are available in the DDBJ/EMBL/GenBank database under the accession numbers AB162141 (ToLCJV-Ageratum) and AB162142 (DNAβ03).

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dicotyledonous plants. However, some begomoviruses do not have a DNA-B component, such as tomato leaf curl virus (ToLCV) [14], tomato leaf curl Karnataka virus (ToLCKV) [8], tomato leaf curl Madagascar virus (ToLCMGV) [12], and tomato leaf curl Philippines virus (ToLCPV) [25]. These viruses have been shown to produce typical disease symptoms when introduced into tomato using a single genomic component.

The first begomovirus satellite DNA, referred to as defective DNAβ, was identified by Dry et al. [13]. Many monopartite begomovirus/DNAβ complexes have since been identified in a wide variety of plant species growing throughout the Old World [2, 4, 22, 26, 36, 37, 43]. DNAβ components have no significant homology with their helper begomoviruses, on which they are dependent for their replication, encapsidation, and movement within and between plants [36].

Tomato leaf curl disease is one of the most damaging tomato diseases worldwide and causes important losses in tomato crop yield [43]. Many viruses that cause leaf curl disease in tomato have been characterized [7, 8, 14, 12, 21, 25, 34, 41]. We have isolated tomato leaf curl Java virus (ToLCJV), Ageratum yellow vein virus-[Java] (AYVV-[Java]), and two satellite DNAβs, DNAβ01 and DNAβ02, from tomato plants in Java, Indonesia, and reported the role of these satellite DNAs in the etiology of begomovirus disease [26]. *A. conyzoides* is a widespread weed that frequently exhibits yellow vein symptoms that have been attributed to begomovirus infection [6, 36]. We have also reported that *A. conyzoides* acts as a reservoir for economically important viral diseases that infect tomato plants in Indonesia [26, 41].

In this paper, we describe the identification and characterization of a begomovirus and a DNAβ isolated from *A. conyzoides* plants with yellow vein disease in Java, Indonesia. The begomovirus has a chimeric genome that may have arisen from recombination between ToLCJV and AYVV-[Java]. We propose to call this virus tomato leaf curl Java virus-Ageratum, and have designated the DNAβ as DNAβ03. We also describe the symptoms induced by ToLCJV-Ageratum and DNAβ03 in *A. conyzoides* and *Nicotiana benthamiana* plants.

Materials and methods

Isolation of a viral genomic DNA and a satellite DNAβ

A. conyzoides plants with yellow vein symptoms were collected in Bandung, West Java, Indonesia, in 2004. Total plant DNA was extracted from the leaf tissue of virus-infected *A. conyzoides* plants and subjected to the polymerase chain reaction (PCR) as described by Kon et al. [25].

Using the begomovirus DNA-A-specific primers UPV1 and UPC 2 [5], a 2.7-kbp DNA fragment was isolated from these plants and sequenced. This viral fragment was found to contain a single *Bam*HI restriction site. Overlapping primers containing a *Bam*HI site were designed to obtain a full-length clone corresponding to the viral genomic DNA-A; the sequences of the primers were 5′-GGATCCACTCGTAA ACGAATTCCCAGAGAC-3′ (AYBam1) and 5′-GGATCC CACATGTTTAAAATAATACTTGG-3′ (AYBam2) (*Bam*HI sites underlined). The putative full-length DNA was amplified and cloned into the pGEM-T Easy vector (Promega) to produce the plasmid pAY1 (ageratum begomovirus).

Two methods were used in an attempt to detect a potential DNA-B component in virus-infected *A. conyzoides* plants. In the first method, we attempted to amplify a DNA-B genomic component using PCR with the primers DNABLC1/DNABLC2 and DNABLV2 [18]. In the second method, we performed PCR using the primers CRv1 (5′-TAATATTACCGGATGGCCGC-3′) and CRc2 (5′-AAAAAATTATGCCA-3′) to attempt to amplify near-full-length DNA fragments representing the genome component(s) of begomoviruses with an intergenic (IR)/common region (CR) sequence similar to that of the ageratum begomovirus. The resulting PCR-amplified fragment was subjected to restriction analysis as described by Kon et al. [25].

The satellite DNA fragment was amplified from total plant DNA using the primers Beta01/Beta02, which were designed to amplify all DNAβ components [3]. The 1.4-kbp PCR product contained a single *Bgl*II site. Overlapping primers containing a *Bgl*II restriction site (underlined), with the sequences 5′-AGATCTGGAAAACGTGAGTGGGCCG AATG-3′ (betaBgl1) and 5′-AGATCTGTTTTGTGTGTGG GGCC-3′ (betaBgl2), were designed in order to amplify a full-length DNAβ fragment. The putative full-length DNAβ was amplified and cloned into pGEM-T Easy to produce the plasmid pAYβ03 (DNAβ).

Sequence analysis

Sequencing was performed with a DYEnamic ET Terminal Cycle Sequencing Kit (Amersham Bioscience) according to the manufacturer's instructions, using either the T7 or SP6 sequencing primers or primers based on the established sequences. The sequencing reactions were resolved using an ABI PRISM 310 Genetic Analyzer (Applied Biosystems). The sequence data were analyzed

using DNASIS Pro version 2.6 (Hitachi Software Engineering). Multiple sequence alignments were created using the optimal alignment method of DNASIS. Phylogenetic and molecular evolutionary analyses comparing the ageratum begomovirus and DNAβ sequences to the sequences of other begomovirus and satellite genomes available in GenBank were generated using the neighbor-joining method in MEGA version 3.0 with 1000 bootstrap repetitions [28].

RDP (recombination detection program) version 2.0 [31] was used to search for recombination events by detecting potential recombined sequences, identifying likely parent sequences, and localizing possible recombination break points. The RDP settings used were multiple comparison correction off, internal reference selection, highest acceptable probability 0.0001, and a window size of 10.

Construction of infectious clones and agroinoculation

Standard methods were used to construct two infectious clones containing partial repeats in the binary vector pBI121 (Clontech) (Fig. 1): a 1.2mer of the begomovirus

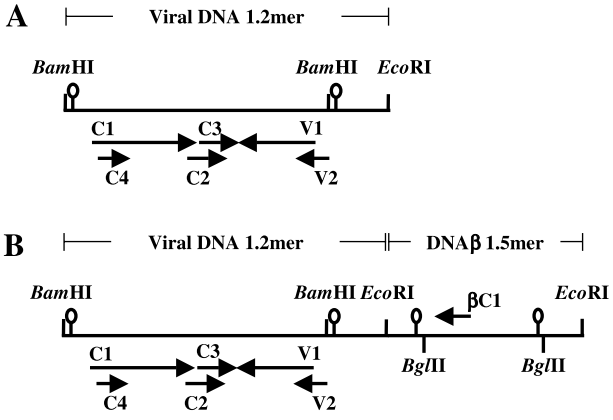

Fig. 1. Construction of infectious clones of **A** the ageratum begomovirus and **B** the ageratum begomovirus and DNAβ. Open circles indicate the stem-loop-forming region. Arrows represent predicted ORFs in both orientations (*C*, complementary; *V*, virion-sense). The restriction sites used in the construction are shown: *Bam*HI (nt 136) and *Eco*RI (nt 2254) in the ageratum begomovirus genomic DNA and *Bgl*II (nt 54) in DNAβ

Table 1. Nucleotide and amino acid sequence identities (%) between the ageratum virus and other begomoviruses

Virus	Total nt	IR nt	LIR nt	RIR nt	V1 aa	V2 aa	C1 aa	C2 aa	C3 aa	C4 aa
AEV	77	68	68	67	82	74	84	62	63	64
AYVCNV-[Hn2]	83	74	72	76	86	76	84	90	92	42
AYVSLV	78	69	69	70	82	71	83	63	70	47
AYVTV-[TW]	84	78	68	88	88	90	76	91	93	47
AYVV	88	78	69	90	87	91	93	91	94	88
AYVV-[Java]	89	**84**	**89**	78	87	90	**95**	88	94	**91**
SbLCV	86	73	72	74	86	94	84	88	88	88
TbLCJV	75	67	74	60	73	68	82	61	62	58
ToLCBDV	78	71	74	68	82	76	80	64	70	51
ToLCBV	75	70	70	70	76	65	78	63	64	50
ToLCJV	**91**	**84**	75	**96**	**98**	**97**	89	**94**	**96**	67
ToLCKV	78	71	70	72	83	76	79	63	66	49
ToLCLV	82	79	75	84	87	68	91	75	69	78
ToLCNDV-Svr	74	65	64	60	81	62	79	49	62	42
ToLCPV	77	60	61	59	77	53	87	68	69	67
ToLCTWV	78	67	63	71	82	69	81	72	70	49
ToLCV	77	65	65	65	80	60	85	65	67	72
TYLCCNV	77	74	71	77	82	70	79	69	69	45
TYLCSV	76	65	66	63	77	70	80	60	60	72
TYLCTHV-[1]	76	72	71	74	78	69	78	71	70	46
TYLCV	75	66	67	65	77	70	81	59	64	78

nt Nucleotide, *aa* amino acid.
IR Intergenic region, *LIR* left intergenic region, *RIR* right intergenic region.
Highest values are in bold.

DNA, either alone (pBAY1.2) or together with a 1.6mer of DNAβ (pBAY1.2β). The *Agrobacterium tumefaciens* strain C58C1 was transformed with the plasmids using the freeze-thaw method [10]. *Ageratum conyzoides* and *N. benthamiana* seedlings were used for agroinoculation experiments. Agroinoculation and viral DNA detection were carried out as described by Kon et al. [24].

Results

Ageratum begomovirus and satellite DNAβ genome organization

The complete nucleotide sequence of the ageratum begomovirus genomic DNA (accession num-

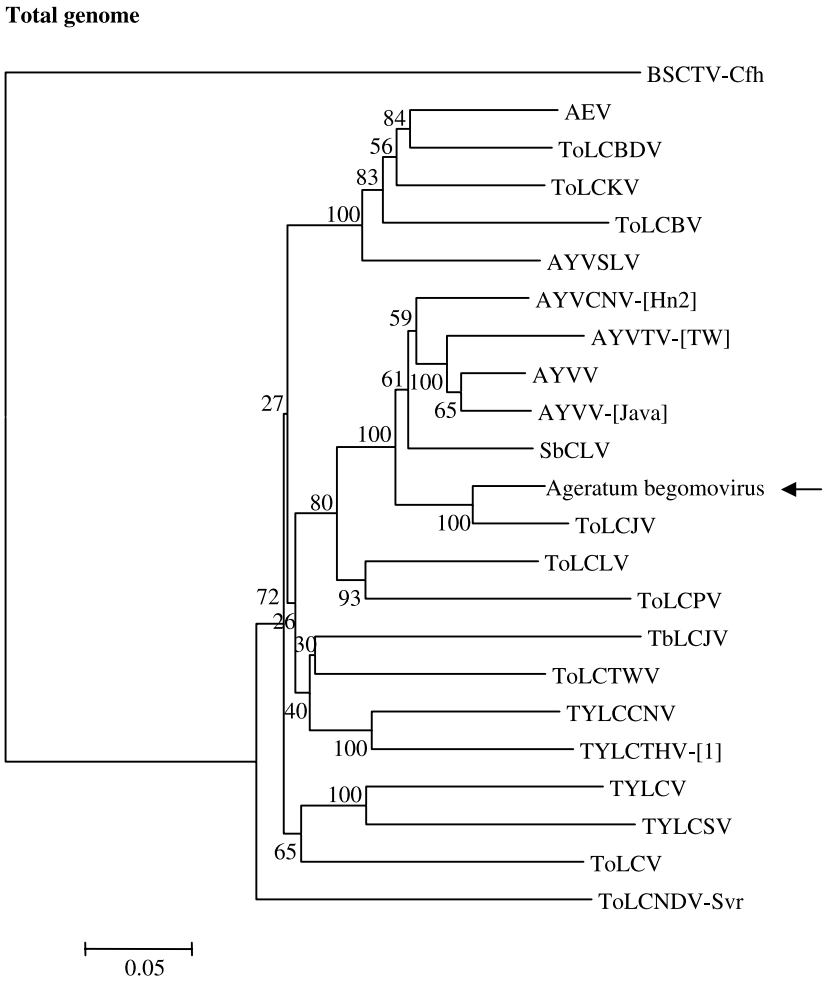

Fig. 2. Neighbor-joining phylogenetic tree produced using begomovirus DNA-A components. The tree was rooted on the genomic sequence of beet severe curly top virus-Cfh (BSCTV-Cfh; accession number U02311) of the genus *Curtovirus*. Vertical distances are arbitrary, and horizontal distances are proportional to the calculated mutation distances. The following sequences were obtained from the GenBank, EMBL or DDBJ databases and used in phylogenetic and comparative analyses: Ageratum enation virus (AEV: AJ437618), Ageratum yellow vein China virus-[Hn2] (AYVCNV-[Hn2]: AJ495813), Ageratum yellow vein Sri Lanka virus (AYVSLV: AF314144), Ageratum yellow vein Taiwan virus-[Taiwan] (AYVTV-[TW]: AF307861), Ageratum yellow vein virus (AYVV: X74516), Ageratum yellow vein virus-[Java] (AYVV-[Java]: AB100305), soybean crinkle leaf virus (SbCLV: AB050781), tobacco leaf curl Japan virus (TbLCJV: AB028604), tomato leaf curl Bangalore virus (ToLCBV: Z48182), tomato leaf curl Bangladesh virus (ToLCBDV: AF188481), tomato leaf curl Java virus (ToLCJV: AB100304), tomato leaf curl Karnataka virus (ToLCKV: U38239), tomato leaf curl Laos virus (ToLCLV: AF195482), tomato leaf curl New Delhi virus-Severe (ToLCNDV-Svr: U15015, DNA-A), tomato leaf curl Philippines virus (ToLCPV: AB050597), tomato leaf curl Taiwan virus (ToLCTWV: U88692), tomato leaf curl virus (ToLCV: S53251), tomato yellow leaf curl China virus (TYLCCNV: AF311734), tomato yellow leaf curl Sardinia virus (TYLCSV: X61153), tomato yellow leaf curl Thailand virus-[1] (TYLCTHV-[1]: X63015, DNA-A), tomato yellow leaf curl virus (TYLCV: X15656)

ber AB162141) consists of 2747 nucleotides. The sequence has the genome organization typical of whitefly-transmitted monopartite begomoviruses from the Old World, which have two open reading frames (ORFs) on the virion-sense strand and four ORFs on the complementary-sense strand that all encode predicted proteins of molecular masses greater than 10 kDa. The two ORFs on the viral strand are designated V1 (coat protein; CP) and V2, and the four ORFs on the complementary strand are designated C1 (replication-associated protein; Rep), C2 (transcriptional activator protein; TrAP), C3 (replication enhancer; REn), and C4. A 33-base region with the potential to form a stem loop (5′-GCGGCCATCCGTATAATATTACCGATGGCC GCG-3′) is present in the intergenic region (IR). This sequence includes the conserved nonanucleotide TAATATTAC, which contains the DNA nicking site for the initiation of viral strand replication in the stem-loop structure. The putative Rep-binding motif GGAGACA is present in the virion-sense strand of the ageratum begomovirus genomic DNA, at nucleotides 2615–2621, 2642–2648, and 2649–2655. Efforts to detect DNA-B components associated with the ageratum begomovirus using both RFLP analysis and PCR using the primers DNABLC1/DNABLC2 and DNABLV2 were unsuccessful.

The ageratum begomovirus DNAβ (accession number AB162142) is 1360 nucleotides in length. This DNAβ contains one ORF (βC1) in the complementary-sense strand that encodes a putative polypeptide of 13.6 kDa (118 amino acids), and an A-rich region (∼65% A) between nucleotides 733 and 946. The putative Rep-binding motif in the begomovirus genome is not present in the DNAβ component, although the similar sequences GGA GAAG (nucleotides 1224–1230), GGTGTGTA (nucleotides 1274–1281), and GGTAGAAA (nucle-

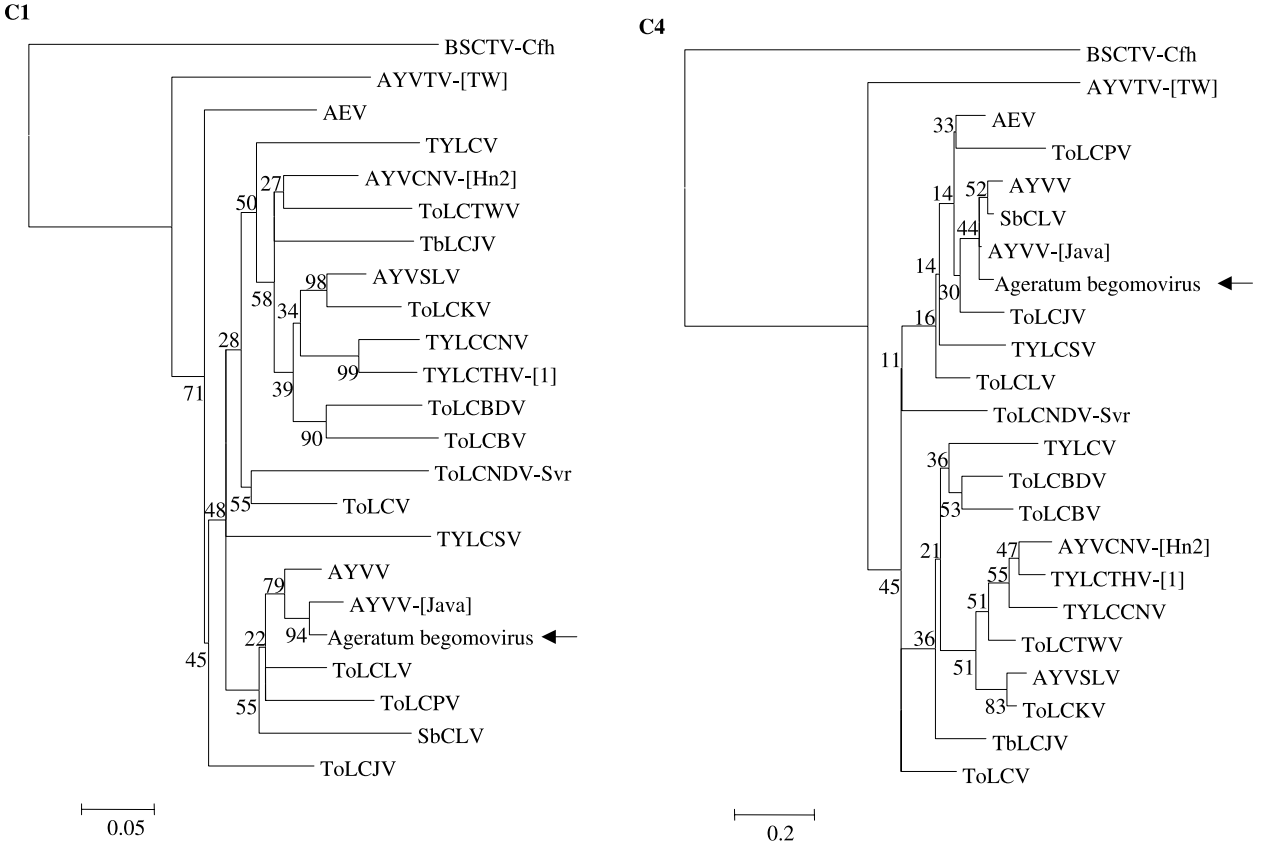

Fig. 3. Neighbor-joining phylogenetic tree generated using the deduced amino acid sequences of begomovirus *C1* and *C4* genes. The tree was rooted on the corresponding sequences in BSCTV-Cfh. Vertical distances are arbitrary, and horizontal distances are proportional to the calculated mutation distances

otides 1292–1299) are located in the virion-sense strand upstream of the stem loop.

Sequence analysis

BLAST searches using the entire DNA sequence revealed that the ageratum begomovirus is most closely related to monopartite begomoviruses from Southeast Asia (Table 1). It shares the highest nucleotide sequence identity (91%) and CP amino acid identity (98%) with ToLCJV. For the V2, C2 and C3 ORF products, the ageratum begomovirus also exhibits the highest amino acid identity (94–97%) with counterparts in ToLCJV. However, for the C1 and C4 ORF products, the ageratum begomovirus shows the highest amino acid identity (95 and 91%, respectively) with counterparts in AYVV-[Java]. The ageratum begomovirus IR has nucleotide identities of 84% with that of AYVV-[Java] and 84% with that of ToLCJV. It shows the highest nucleotide identity in the left IR (LIR) with AYVV-[Java], at 89%, whereas that of the right IR (RIR) is most similar to that from ToLCJV, at 96%.

A phylogenetic tree was created using the nucleotide and amino acid sequences of begomoviruses from the Old World. A phylogenetic analysis of the DNA-A component sequences resulted in the ageratum begomovirus clustering with ToLCJV (Fig. 2). A similar relationship appeared in an analysis of the ageratum begomovirus CP sequence (data not shown). In contrast, phylogenetic analyses of both the C1 and C4 amino acid sequences resulted in the ageratum begomovirus clustering with AYVV-[Java] (Fig. 3).

Using a recombination detection program (RDP), we examined the possibility that ageratum begomovirus has a chimeric genome that arose from a recombination event between those of ToLCJV and AYVV-[Java]. The RDP analysis provided evidence of recombination between these two viruses. In this recombination event, a region of the ToLCJV genome (from nucleotides 2389 to 2692) was replaced

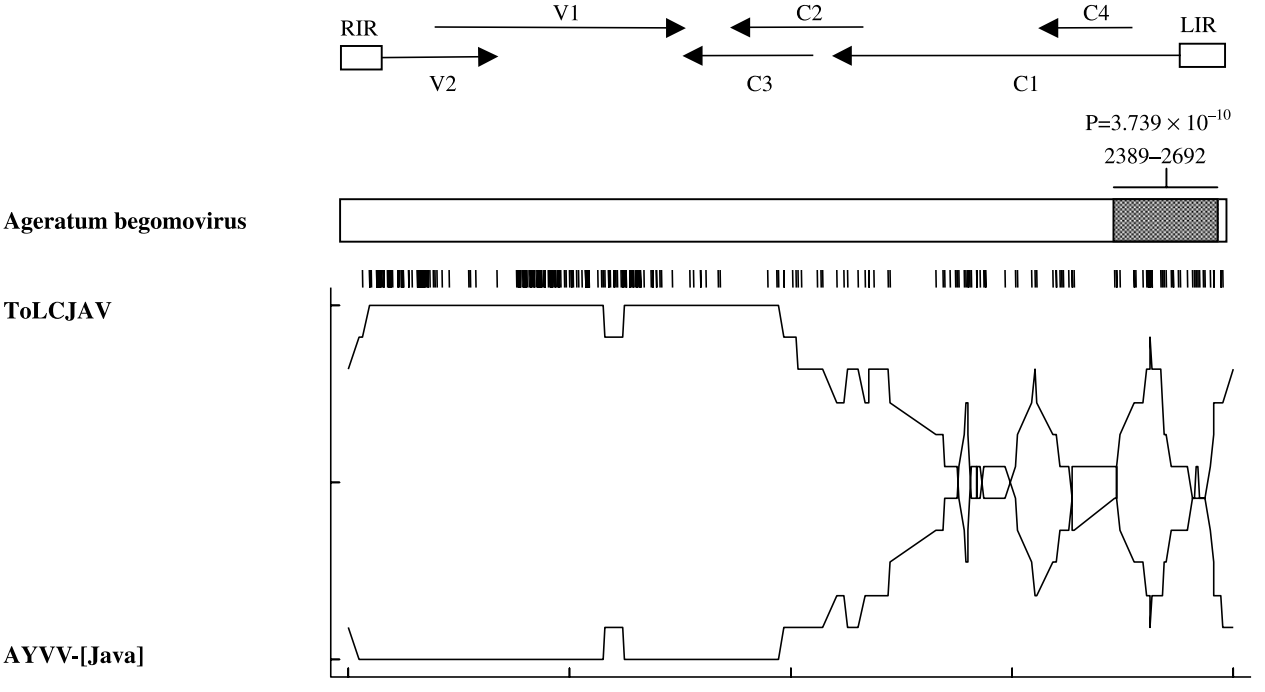

Fig. 4. Schematic representation of the recombinant regions in the ageratum begomovirus genomic DNA. Potential recombination break points and the probabilities (*P*) that the indicated regions do not have a recombination origin are presented. Shading patterns indicate the viral origin of the recombinant region. The y-axis illustrates pairwise identity, and the putative recombinant region is shown by the crossover of the panel. The probability that the sequences at this crossover could appear recombinant simply due to chance convergence is shown (3.739×10^{-10})

with the corresponding AYVV-[Java] genomic sequence (RDP P-value $= 3.739 \times 10^{-10}$), which includes the 5′ portion of the IR, C4 and C1 ORFs (Fig. 4). ToLCJV was identified as a major parent of ageratum begomovirus, and AYVV-[Java] was identified as a minor parent. The ageratum virus clearly has a backbone of the ToLCJV genome. According to the International Committee on Taxonomy of Viruses (ICTV) criteria for begomovirus species demarcation using DNA-A nucleotide and CP amino acid sequences [15], these results indicate that the ageratum begomovirus is a strain of ToLCJV, for which the name tomato leaf curl Java virus-Ageratum (ToLCJV-Ageratum) is proposed.

The sequence of the ToLCJV-[Ageratum] DNAβ has the greatest percentage identity (91 and 90%, respectively) with those of DNAβ01 and DNAβ02 (Kon et al., 2006), which are associated with ToLCJV and AYVV-[Java], and has a lower percentage identity (82%) with that of AYVV DNAβ. The DNAβ βC1 product has a high amino acid identity with its counterparts in ToLCJV and AYVV-[Java] DNAβ01 (94%) and DNAβ02 (87%), and a lower amino acid identity with its counterpart in AYVV DNAβ (77%). ToLCJV-Ageratum DNAβ has no obvious sequence homology in the ToLCJV-Ageratum genome, except for the nonanucleotide TAATATTAC. A phylogenetic tree was constructed based on an alignment of the complete ToLCJV-Ageratum DNA nucleotide sequence and those of selected DNAβs associated with other monopartite begomoviruses (Fig. 5). ToLCJV-Ageratum DNAβ clusters with ToLCJV and AYVV-[Java] DNAβ01 and DNAβ02, which were isolated from

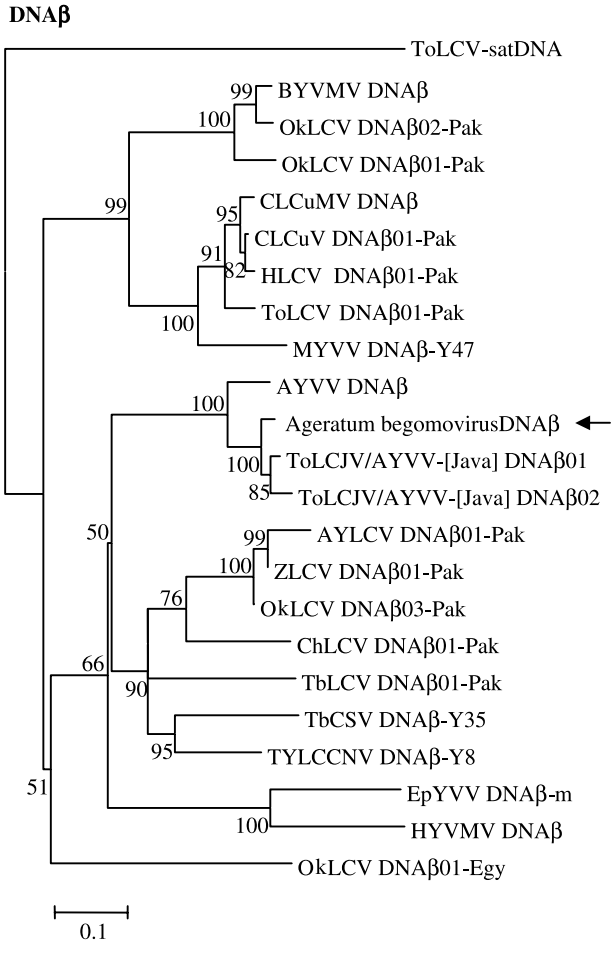

Fig. 5. A neighbor-joining phylogenetic tree generated using the complete sequences of begomovirus DNAβ components. The tree was rooted on the sequence of the tomato leaf curl virus satellite DNA (ToLCV stDNA; accession number U74627), a sequence distinct from those of other DNAβs associated with begomoviruses. Vertical distances are arbitrary, and horizontal distances are proportional to the calculated mutation distances. The following sequences were obtained from the GenBank, EMBL or DDBJ databases and used in phylogenetic and comparative analyses: Ageratum yellow leaf curl virus DNAβ01-Pakistan (AYLCV DNAβ01-Pak: AJ316026), AYVV DNAβ (AJ252072), Bhendi yellow vein mosaic virus DNAβ (BYVMV DNAβ: AJ308425), chilli leaf curl virus DNAβ01-Pak (ChLCV DNAβ01-Pak: AJ316032), cotton leaf curl Multan virus DNAβ (CLCuMV DNAβ: AJ292769), cotton leaf curl virus DNAβ01-India (CLCuVβ01-Ind: AJ316038), Eupatrium yellow vein virus DNAβ (EpYVV DNAβ-m: AJ438938), Hibiscus leaf curl virus DNAβ01-Pakistan (HLCV DNAβ01-Pak: AJ297908), honeysuckle yellow vein virus DNAβ (HYVMV DNAβ: AJ316040), Malvastrum yellow vein virus DNAβ (MYVV DNAβ-Y47: AJ421482), okra leaf curl virus DNAβ01-Egypt (OkLCV DNAβ01-Egy: AF397217), okra leaf curl virus DNAβ01-Pakistan (OkLCV DNAβ01-Pak: AJ316029), okra leaf curl virus DNAβ02-Pakistan (OkLCV DNAβ02-Pak: AJ316030), okra leaf curl virus DNAβ03-Pakistan (OkLCV DNAβ03-Pak: AJ316031), tobacco curly shoot virus-Y35 DNAβ (TbCSV DNAβ-Y35: AJ421484), tobacco leaf curl virus DNAβ01-Pakistan (TbLCV DNAβ01-Pakistan: AJ316033), ToLCJV/AYVV-[Java] DNAβ01 and DNAβ02 (AB100306 and AB113651), tomato leaf curl virus DNAβ01-Pakistan (ToLCV DNAβ01-Pak: AJ316035), TYLCCNV DNAβ-Y8 (AJ421622) and Zinnia leaf curl virus DNAβ01-Pakistan (ZLCV DNAβ01-Pak: AJ316041)

Indonesian tomato plants showing leaf curl disease. ToLCJV-Ageratum DNAβ also clusters with AYVV DNAβ but is only distantly related to other DNAβs. These results show that the relatedness of the ToLCJV-Ageratum DNAβ with other similar entities is linked to the geographic origins of these molecules. Based on these results, we proposed the name ToLCJV-Ageratum DNAβ03 for this DNAβ.

Infectivity and symptoms induced by ToLCJV-Ageratum and DNAβ03

The infectivity and symptoms caused by ToLCJV-Ageratum and DNAβ03 in *A. conyzoides* and *N. benthamiana* were examined (Table 2). Plants mock inoculated with *Agrobacterium* containing the empty binary vector did not develop symptoms (Fig. 6a). When introduced into *A. conyzoides* plants by agroinoculation, ToLCJV-Ageratum alone (clone pBAY1.2) infected the plants systemically but caused no symptoms (Fig. 6b). However, co-inoculation of ToLCJV-Ageratum and DNAβ03 (clone pBAY1.2β03) resulted in typical yellow vein symptoms at 28 days post-inoculation (dpi) (Fig. 6c). No symptoms were observed on *A. conyzoides* plants agroinoculated with DNAβ03 alone. The presence of viral genomic DNA and DNAβ in agroinoculated plants was confirmed by Southern blot analysis (data not shown).

N. benthamiana plants agroinoculated with ToLCJV-Ageratum alone were systemically infected

Table 2. Infectivity of ToLCJV-Ageratum and DNAβ03 by *Agobacterium*-mediated inoculation

Plant	Inoculum[a]	Infectivity[b]	Symptoms
Ageratum conyzoides	pBI121	0/24 (3)	no symptoms
	pBAY1.2	5/24 (3)	no symptoms
	pBAY1.2β	20/24 (3)	yellow vein
	pBAYβ	0/24 (3)	no symptoms
Nicotiana benthamiana	pBI121	0/15 (3)	no symptoms
	pBAY1.2	15/15 (3)	severe upward leaf curl, vein-swelling and stunting
	pBAY1.2β	15/15 (3)	severe downward leaf curl and stunting
	pBAYβ	0/24 (3)	no symptoms
	pBToX1.4	15/15 (3)	mild downward leaf curl
	pBToB1.4	15/15 (3)	severe upward leaf curl, vein-swelling and stunting

[a] pBAY1.2, ToLCJAV-Ageratum; pBAY1.2β, ToLCJAV-Ageratum + DNAβ03; pBAYβ, DNAβ03. Infectious clones of pBToX1.4 (ToLCJV) and pBToB1.4 (AYVV-[Java]) were previously constructed by Kon et al. [26].
[b] Number of infected plants/number of plants inoculated (number of experiments).

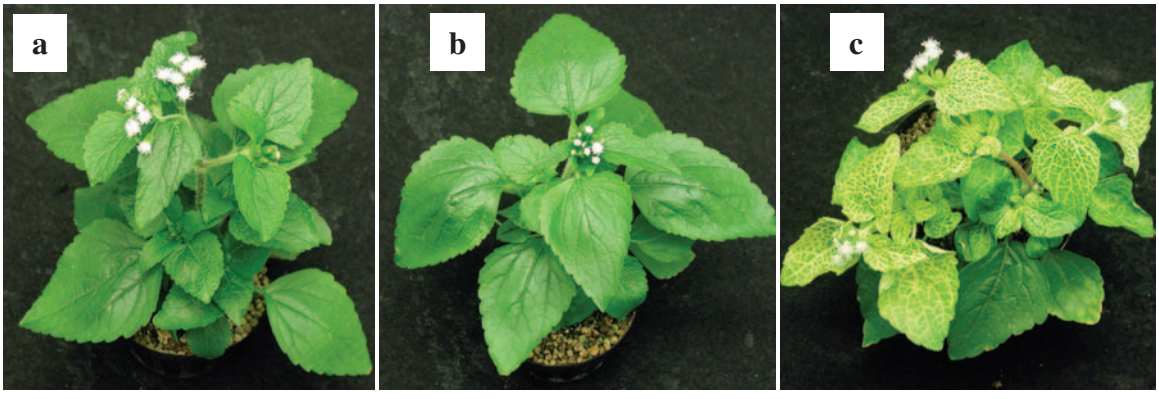

Fig. 6. *A. conyzoides* plants either (**a**) mock-inoculated or inoculated with (**b**) ToLCJV-Ageratum alone, or (**c**) ToLCJV-Ageratum and DNAβ03. The plants were photographed at 28 days post-inoculation

and developed severe upward leaf curl, vein-swelling, and stunting symptoms. Agroinoculation of *N. benthamiana* with ToLCJV induced mild downward leaf curl symptoms, whereas AYVV-[Java] induced severe upward leaf curl, vein-swelling, and stunting symptoms. The symptoms that ToLCJV-Ageratum caused in *N. benthamiana* were similar to those caused by AYVV-[Java] but differed from those caused by ToLCJV. *N. benthamiana* plants co-agroinoculated with ToLCJV-Ageratum and DNAβ03 developed severe downward leaf curl and stunting symptoms.

Discussion

We have detected a new recombinant begomovirus that infects *A. conyzoides* plants in Indonesia and is accompanied by a satellite DNAβ. We isolated and sequenced the full-length genomes of both agents. Phylogenetic and genetic recombination analyses demonstrated that the weed-infecting begomovirus is a monopartite begomovirus typical of the eastern hemisphere and has a high identity to ToLCJV, except for the region corresponding to the C1 and C4 ORFs and the origin of replication, which show a higher sequence identity with the AYVV-[Java] genome. Comparison of the nucleotide and amino acid sequences with those of other begomoviruses revealed that the ageratum begomovirus is a strain of ToLCJV, for which we propose the name tomato leaf curl Java virus-Ageratum (ToLCJV-Ageratum). The satellite DNAβ, isolated from *A. conyzoides* infected with ToLCJV-Ageratum has high sequence identity to DNAβs from tomato plants, previously characterized by Kon et al. [26].

Agroinoculation experiments with ToLCJV-Ageratum alone demonstrated that the virus can replicate autonomously and move systemically in *A. conyzoides* plants but does not induce yellow vein symptoms. However, in the presence of DNAβ03, ToLCJV-Ageratum does induce these symptoms, which appear similar to those observed in the field in Java, Indonesia. Therefore, the ageratum yellow vein disease complex comprises ToLCJV-Ageratum and DNAβ03. The requirement that both a begomovirus and its associated satellite DNAβ be present for typical disease symptoms to occur has been demonstrated for cotton leaf curl, Bhendi yellow mosaic, ageratum yellow vein, tomato leaf curl, and tomato yellow leaf curl diseases [4, 22, 26, 36, 43]. DNAβ molecules encode a highly conserved ORF, βC1, and are essential for symptom induction [11, 38]. We demonstrated that DNAβ03 is replicated by ToLCJV-Ageratum. A putative Rep-binding motif (termed iteron [9, 19, 29]), present in several instances in the ToLCJV-Ageratum genome is not present in DNAβ03, but the DNAβ03 sequence contains a putative iteron sequence located upstream of the stem-loop structure that is identical to the iteron sequence of DNAβ02. However, the mechanism of Rep-mediated replication of DNAβ has not yet been resolved. The DNAβ03 sequences does not contain the iterons of the ToLCJV-Ageratum genome, suggesting a more relaxed specificity for Rep binding during begomovirus replication. The satellite DNAβ appears to be capable of being replicated by Reps from diverse monopartite and bipartite begomoviruses [1, 2, 35, 39].

A. conyzoides is a weed that often grows near tomato fields in Southeast Asia, including Indonesia, and frequently exhibits yellow vein symptoms [6, 41]. *A. conyzoides* could be a reservoir for begomoviruses that cause tomato leaf curl disease [26]. Inoculation of *N. benthamiana* plants with ToLCJV-Ageratum alone produced severe upward leaf curl, vein swelling, and stunting symptoms. However, in the presence of DNAβ03, ToLCJV-Ageratum induced leaf downward curl and stunting symptoms in *N. benthamiana* plants. Consistent with these results, a change from upward to downward leaf curl in the presence of DNAβ has been demonstrated experimentally for AYVV in *N. benthamiana* plants [30, 38]. Our results further support the hypothesis that the DNAβ plays an important role in symptom induction. We previously showed that ToLCJV and AYVV-[Java] produce a symptomatic infection in *N. benthamiana* [26]. ToLCJV alone induces mild downward leaf curl symptoms, whereas AYVV-[Java] induces severe upward leaf curl, vein swelling, and stunting. Thus, ToLCJV-Ageratum induces symptoms similar to those caused by AYVV-[Java] and probably evolved by acquiring a pathogenicity determinant from AYVV-[Java]. The *C4* gene has been shown to

be responsible for this leaf curl phenotype for geminiviruses such as beet curly top virus and tomato leaf curl virus [27, 40].

The generation of genetic diversity in viral populations facilitates adaptation to new hosts and changing environmental conditions. Three major forces drive the evolution of viruses: mutation, recombination, and reassortment [20]. It is generally accepted that recombination plays an important role in the evolution and genetic diversification of emerging begomovirus populations [16, 17, 23, 32]. We have previously demonstrated that tomato leaf curl disease is caused by either the ToLCJV/DNAβ02 or the AYVV-[Java]/DNAβ02 complexes [26]. In the present study, we found evidence for interspecies recombination between ToLCJV and AYVV-[Java]. Because these viruses have common hosts, such as tomato and *A. conyzoides*, mixed infection can occur and viruses may replicate simultaneously in the same cell, a prerequisite for recombination [33]. These results emphasize the potential for the emergence of novel begomoviruses by interspecies genetic recombination.

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