CAPS Analysis: A Possible Tool to Detect and Group Geminiviruses Infecting Some Fibre Crops and Weeds

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Abstract

Some fibre crops and weeds, showing typical symptoms caused by begomoviruses, were subjected to PCR amplification using different sets of primers specific for DNA A and DNA β molecules of begomoviruses. Restriction digestion of full length DNA A and DNA β using different enzymes revealed variation in restriction profiles amongst the virus isolates in the present study. Moreover, variation in the restriction profile of different DNA β molecule with that of their corresponding reported sequences were also noticed. Cleaved amplified polymorphic sequence (CAPS) analysis of DNA β revealed little or no genetic distance between the begomoviruses infecting *Sida acuminata* and *S. rhombifolia* and also among the begomoviruses infecting *Urena*, *Hibiscus cannabinus*, and *H. sabdariffa*. But the same analysis with full length DNA A showed close phylogenetic relationships between begomoviruses infecting *H. cannabinus* and *H. sabdariffa*. Hence, for wide spread variation and unavailability of any sequence information CAP analysis might be a useful tool to detect and group begomoviruses.

Introduction

Newly emerging geminiviruses are causing severe disease epidemics in cotton, grain, legumes, tomato, and other staple food and cash crops in tropical and subtropical regions (1,13). These viruses cause a variety of symptoms including vein yellowing, yellow mosaic, and leaf curl and are spreading at an alarming pace due to a high rate of recombination (2,15). Control of the spread of these viruses is difficult because they are transmitted through insect vectors like whitefly. In addition, several weeds serve as alternate hosts for these viruses in the absence of the main crops.

The majorities of the geminiviruses infecting dicotyledonous plants belong to the genus *Begomovirus* and contain single-stranded bipartite DNA genome, referred to as DNA A and DNA β , which are approximately 2.7 kb in total length (19). Monopartite begomoviruses lack the DNA β component and for these viruses all the viral genes required for replication, gene expression, whitefly-transmission and systemic infection are encoded on a single DNA component which is homologous to the DNA A component of bipartite begomoviruses (16,18). Some satellite DNA β molecules are associated with such monopartite begomoviruses (3,5,6,7,8,9,10,20). These satellite molecules depend upon their helper viruses for replication, movement, and insect-transmission and alter the symptom production in some host plants (3,20). Furthermore, such satellite DNA β have been reported to be co-evolved with their cognate helper viruses (23). Different agro-ecological situations under which plants with multiple infections of different begomoviruses and their satellites might be a suitable condition for recombination producing new strains. With an aim to diagnose such newer emerging geminiviruses and to understand their relationships, we searched for plants showing symptom expressions like yellow vein mosaic, yellowing of leaves, leaf curl, and leaf mosaic, generally caused by geminiviruses,

in some economically important fibre crops and their neighboring weeds. Southern hybridization technique and nucleic acid spot hybridization tests using probes specific for DNA β (accession no. DQ298137) and coat protein gene of DNA A (DQ298138) of *Mesta yellow vein mosaic virus* (5) also indicated the presence of begomoviruses in various test plants in previous study (10). The variability among the isolates with the reported ones was judged by CAPS analysis in the present study.

DNA Isolation and PCR Amplification

Fiber crops and weed plants, located in close proximity to the main crops, showing typical geminiviral symptoms were used in the present investigation (Table 1). The infected plants were collected from multiple localities and maintained by whitefly transmission at an insect-free glasshouse in controlled temperature and humidity for experimental study.

Table 1. PCR amplification of plant samples used in this study showing different symptom expressions.

Name of the plants ^W	Family	Symptoms	PCR amplification			
			Satellite DNA β	DNA A		
				Full length	Coat protein	Degenerate primer ^Z
<i>Croton bonplandianum</i>	Euphorbiaceae	Yellow vein mosaic	+	—	+	+
<i>Sida acuminata</i>	Malvaceae	Yellow vein mosaic	+	—	+	+
<i>S. rhombifolia</i>	Malvaceae	Yellow vein mosaic	+	—	+	+
<i>Ageratum conyzoides</i>	Asteraceae	Yellow vein mosaic	+	—	—	+
<i>Lycopersicon esculentum</i>	Solanaceae	Leaf curl	+	+ ^x	+	+
<i>Abelmoschus esculentus</i>	Malvaceae	Yellow vein mosaic	+	+ ^y	+	+
<i>Urena lobata</i>	Malvaceae	Yellow vein mosaic	+	+ ^y	+	+
<i>Hibiscus cannabinus</i>	Malvaceae	Yellow vein mosaic	+	+ ^x	+	+
<i>H. sabdariffa</i>	Malvaceae	Yellow vein mosaic	+	+ ^x	+	+
<i>Nicotiana tabacum</i>	Solanaceae	Leaf curl	—	+ ^x	+	+
<i>Acalypha indica</i>	Euphorbiaceae	Yellowing of leaf	—	—	+	+
<i>Clerodendrum indicum</i>	Verbenaceae	Yellowing of leaf	—	—	+	+
<i>Dolichos lablab</i>	Fabaceae	Golden mosaic	—	—	+	+
<i>Carica papaya</i>	Caricaceae	Leaf curl	—	+ ^x	—	+
<i>Corchorus capsularis</i>	Tiliaceae	Mosaic	—	—	—	+
<i>Clitoria turnatea</i>	Fabaceae	Yellow mosaic	—	—	—	+
<i>Hemidesmus fluviroidis</i>	Scrophulariaceae	Yellowing of leaf	—	—	—	+

(continued)

Table 1. (continued)

Name of the plants ^w	Family	Symptoms	PCR amplification			
			Satellite DNA β	DNA A		
				Full length	Coat protein	Degenerate primer ^z
<i>Eclipta alba</i>	Asteraceae	Yellow vein mosaic	—	—	+	—
<i>Vigna mungo</i>	Fabaceae	Golden mosaic	—	—	+	—
<i>Gomphrena globosa</i>	Amaranthaceae	Yellow vein mosaic	—	—	—	—
<i>Abutilon indica</i>	Malvaceae	Yellow vein mosaic	—	—	—	—
<i>Malachra capitata</i>	Malvaceae	Yellow vein mosaic	—	—	—	—
<i>Hibiscus moschata</i>	Malvaceae	Yellow vein mosaic	—	—	—	—
<i>Sida acuta</i>	Malvaceae	Leaf curl and yellow vein mosaic	—	—	—	—
<i>Corchorus aestuans</i>	Tiliaceae	Yellow vein mosaic	—	—	—	—
<i>Vernonia tarnata</i>	Scrophulariaceae	Yellow vein mosaic	—	—	—	—

^w Plants collected from multiple (more than six collections) localities.

^x Amplified with FLD-F and FLD-R primer sets (designed in this study).

^y Amplified with Bcp1.f and Bcp2.r primer sets (21).

^z Amplified with Primer A and Primer B primer sets (11).

Total DNA from the leaf tissues of the infected plants were isolated using DNeasy Plant Mini Kit (QIAGEN). PCR amplification of DNA was done by using 5 sets of geminivirus specific primers (2,11,21) and degenerate primers designed in this study (FLD-F: 5'- GARAGTACYCATGCGYTCTAAYCC -3', FLD-R: 5'- AGTRTGRTTYTCRTACTTCCAG - 3'). In all the PCR experiments template DNA from healthy plants served as negative controls.

Of the 26 plants tested, 19 were successfully amplified with one or more primer sets having variable amplicon sizes (Fig. 1 and Table 1). No PCR amplicon was produced using the DNA extracts obtained from control plants of each type. Isolates obtained from plants showing positive amplification showed two major groups: begomoviruses with satellite DNA β and begomoviruses without satellite DNA β molecule.

Fig. 1. PCR amplicon of different plant samples: (a) full length DNA A (2.7 kb); (b) coat protein part of DNA A (0.77 kb); (c) common region and part of coat protein part of DNA A (0.5 kb); and (d) full length DNA β (1.3 kb).

DNA used in the PCR from the host: *Croton* (lane 1); *Sida acuminata* (lane 2); *S. rhombifolia* (lane 3); *Ageratum* (lane 4); *Lycopersicon* (lane 5); *Abelmoschus* (lane 6); *Urena* (lane 7); *Hibiscus cannabinus* (lane 8); *H. sabdariffa* (lane 9); *Nicotiana* (lane 10); *Acalypha* (lane 11); *Clerodendron* (lane 12); *Dolichos* (lane 13); *Carica* (lane 14); *Corchorus capsularis* (lane 15); *Clitoria* (lane 16); *Hemidesmus* (lane 17); *Eclipta* (lane 18); and *Vigna* (lane 19). M = 1 kb DNA ladder (Fermentas, USA).

The present study revealed that PCR techniques using specific and degenerate primers could be used as a diagnostic tool to rapidly screen plants for geminiviruses. We found the degenerate primers were more robust than the other primer pairs (4,11,17) tested in the PCR assays. The broad detection range of the degenerate primers may therefore aid in the detection of other uncharacterized geminiviruses. Besides virus detection, the methods used in the study have offered means of distinguishing and grouping viruses. The results also indicate the association of satellite DNA β molecules with a number of begomovirus isolates in West Bengal, India.

CAPS Analysis of Different DNA A and DNA β Molecules

CAPS analysis of 7 full length DNA A (Fig. 2) and 9 satellite DNA β amplicons (Fig. 3) was performed using 7 restriction enzymes (*Eco*R1, *Taq*I, *Mse*I, *Alu*I, *Hae*II, *Rsa*I, and *Hpa*II). The CAPS-generated fingerprints were

further analyzed for understanding the phylogenetic relationship among them. A dendrogram illustrating phylogenetic relationships based on CAPS analysis of different DNA A and satellite DNA β was prepared by using NTSYSpc version 2.20e (Applied Biostatistics Inc.). A similarity matrix using pair wise Jaccard's similarity coefficients (12) was used in cluster analysis using UPGMA and SHAN clustering algorithms to obtain the dendrogram.

Fig. 2. CAPS Analysis of different full length DNA A using restriction enzyme (a) *EcoRI*, (b) *TaqI*, (c) *MseI*, (d) *AluI*, (e) *HaeIII*, (f) *RsaI*, and (g) *HpaII*. PCR amplicon used: lane 1, *Hibiscus sabdariffa*; lane 2, *H. cannabinus*; lane 3, *Lycopersicon esculentum*; lane 4, *Nicotiana tabacum*; lane 5, *Carica papaya*; lane 6, *Urena lobata*; and lane 7, *Abelmoschus esculentus*. M = 1 kb DNA ladder (Fermentas, USA).

Fig. 3. CAPS Analysis of different DNA β using restriction enzyme (a) *EcoR1*, (b) *Taq1*, (c) *Mse1*, (d) *Alu1*, (e) *HaeIII*, (f) *RsaI*, and (g) *HpaII*. PCR amplicon used: lane 1, *Croton bonplandianum*; lane 2, *Sida acuminata*; lane 3, *S. rhombifolia*; lane 4, *Ageratum conyzoides*; lane 5, *Lycopersicon esculentum*; lane 6, *Abelmoschus esculentus*; lane 7, *Urena lobata*; lane 8, *Hibiscus cannabinus*; and lane 9, *H. sabdariffa*. M = 1 kb DNA ladder (Fermentas, USA).

The reported sequence based restriction sites of other isolates of begomoviruses infecting the same hosts under study were compared with those of the isolates in the present study. Such comparisons with DNA A restriction profiles indicated no variation, while variations in the restriction sites with that of reported sequences was observed in the restriction profile of satellite DNA β molecules obtained from infected plants like *Croton*, *Sida*, *Ageratum*, *Lycopersicon*, and *Abelmoschus*. The results revealed the presence of an *EcoR1* site in the DNA β of begomovirus isolates infecting *Croton*, whereas this site was absent in the reported sequence of begomovirus infecting *Croton* (accession no. AJ968684). The Indian isolate of begomovirus infecting *Ageratum*, showed the presence of *EcoR1* and *Taq1* sites in the satellite DNA β molecule, while these sites were absent in the China isolate of *Ageratum yellow vein mosaic virus* (NC_007067). Some other restriction enzyme sites which were found in the reported sequences have been found absent in the present isolates of different begomoviruses. The China isolate of begomovirus infecting *Sida acuta* (AJ810095) possesses a *HpaII* site in the satellite DNA β , but this was absent in the begomoviruses infecting *Sida acuminata* and *S. rhombifolia* in the present study. Analysis of restriction profiles also revealed the absence of an *Alu1* site in the satellite DNA β of begomovirus isolate infecting tomato in the present study, which was present in the New Delhi virus isolate of *Tomato leaf curl virus* (NC_005359). Likewise, an *RsaI* site contained by the Tamil Nadu isolate of *Bhendi yellow vein mosaic virus* (AJ308425) was missing in the presently used isolate (Fig. 3).

The restriction profile of amplified DNA A did not distinguish begomoviruses infecting, *H. cannabinus* and *H. sabdariffa* in all the restriction enzymes tested.

The restriction profile of satellite DNA β of begomoviruses infecting two species of *Sida*, *S. acuminata*, and *S. rhombifolia* showed similar patterns with begomoviruses infecting *Urena lobata*, *H. cannabinus*, and *H. sabdariffa*.

CAPS is one of the latest marker system for analysis of genomic diversity which involves restriction digestion of same sized amplified product from different sources for generating a restriction profile of the amplicons. The results of the present study detected the polymorphic variability and diversity of DNA A and satellite DNA β molecules in the sample by measuring the size of the restriction fragments and their number in different begomoviruses infecting plants. Regarding the full length DNA A, the restriction profile showed similar types of fragment length polymorphism as had been noted with the reported sequences of begomoviruses infecting *Lycopersicon*, *Nicotiana*, *Carica*, and *Abelmoschus*. The small variation noted during the CAPS analysis could hence be due to mutation, recombination, or pseudo-recombination, a common phenomenon with begomoviruses (14,22). In the case of satellite DNA β , the similar patterns of restriction profiles of begomoviruses obtained from different hosts like *Urena lobata*, *H. cannabinus*, and *H. sabdariffa* indicate close sequence similarity of the DNA β of begomoviruses infecting those crops (6).

CAPS Based Relationship and Grouping of Begomoviruses Under Study

The relationship among the begomoviruses under study has been analyzed with the combined profiles of all the bands resolved in CAPS analysis of full length DNA A and satellite DNA β amplicons. The dendrogram constructed with the CAPS profile analysis of DNA A (Fig. 4) revealed two primary groups: one including begomovirus infecting *H. sabdariffa*, *H. cannabinus*, *Lycopersicon*, *Nicotiana*, and *Carica*; the second group including only begomovirus infecting *Urena* and *Abelmoschus*. In the first group, DNA A restriction profiles of begomoviruses infecting *H. sabdariffa* and *H. cannabinus* showed no differences in phylogenetic relationships and clustered with begomovirus infecting *Nicotiana*, indicating close relationships between them. Again, in the second group, restriction profiles of DNA A of begomoviruses infecting *Lycopersicon* and *Carica* showed a common ancestry and greater genetic distance with the other members of the same group.

Fig. 4. Cladogram illustrating phylogenetic relationships based on CAPS analysis of different DNA A (drawn by SHAN clustering UPGMA method using Jaccard coefficient in NTSYS PC 2.02e software). The begomoviruses isolated from the hosts are: (A) *Hibiscus sabdariffa*; (B) *H. cannabinus*; (C) *Lycopersicon esculentum*; (D) *Nicotiana tabacum*; (E) *Carica papaya*; (F) *Urena lobata*; and (G) *Abelmoschus esculentus*.

Fig. 5. Cladogram illustrating phylogenetic relationships based on CAPS analysis of different DNA β (drawn by SHAN clustering UPGMA method using Jaccard coefficient in NTSYS PC 2.02e software). The begomoviruses isolated from the hosts are: (A) *Croton bonplandianum*; (B) *Sida acuminata*; (C) *S. rhomboidifolia*; (D) *Ageratum conyzoides*; (E) *Lycopersicon esculentum*; (F) *Abelmoschus esculentus*; (G) *Urena lobata*; (H) *Hibiscus cannabinus*; and (I) *H. sabdariffa*.

The satellite DNA β clustered into two primary groups: one including begomoviruses infecting *Croton*, *Ageratum*, and two species of *Sida*; and the second group including begomoviruses infecting *Lycopersicon*, *Abelmoschus*, *Urena*, *H. cannabinus*, and *H. sabdariffa*. In the first group, begomoviruses infecting two species of *Sida*, *S. acuminata* and *S. rhomboidifolia* showed no differences in CAPS and thus confirmed the close origin of the satellite DNA β associated with these hosts. Similarly, in the second group, begomoviruses infecting *Urena*, *H. cannabinus*, and *H. sabdariffa* (from the same family, *Malvaceae*) also showed no differences and formed a cluster in the dendrogram.

Thus, in the present study, the grouping of begomoviruses based on CAPS analysis revealed that the isolates obtained from the same family *Malvaceae* shared a common phylogenetic relationship, which appears to be in line with the fact that the satellite DNA β molecule of begomoviruses showed greater similarity among related hosts than with begomoviruses infecting distantly related hosts (2). Furthermore, the variability of satellite DNA β molecules with yellow vein mosaic disease of *Urena lobata*, *Croton bonplandianum*, *Sida acuminata*, and *S. rhombifolia* indicated the wide spread variation of geminiviruses in different host plants. Hence, the present study indicated that the CAPS technique might be a very useful tool for identification and grouping of begomoviruses especially when sequence information is not available.

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