



Rapid Communication

A novel class of DNA satellites associated with New World begomoviruses

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ARTICLE INFO

Article history:

Received 20 December 2011

Returned to author for revision 11 January 2012

Accepted 18 January 2012

Available online 11 February 2012

Keywords:

Geminiviridae

Begomoviruses

DNA satellites

Weeds

Malvaceae

ABSTRACT

Begomoviruses (family *Geminiviridae*) are whitefly-transmitted plant DNA viruses that have been shown to be helper viruses for a number of distinct DNA satellites, including betasatellites and alphasatellites. Replication of the satellites interferes to some degree with replication of the helper and in some cases they affect the disease symptoms. To date, betasatellites and related molecules such as the satellite associated with *Tomato leaf curl virus* (ToLCV-sat), have only been associated with Old World begomoviruses. Here, we report the discovery and characterization of subviral molecules associated with bipartite begomoviruses from the New World, which constitute a novel class of DNA satellites, in two malvaceous plant species. These molecules, in addition to sharing some genetic features with betasatellites and ToLCV-sat, contain nucleotide stretches of begomoviral origin, presumably the remains of recombination events involved in their origin.

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Introduction

Some isolates of certain plant viruses contain nucleic acids other than genomic, subgenomic or defective nucleic acids, which lack genes that would encode the enzymes required for their replication. Two major classes of such satellite agents can be distinguished according to the source of the coat protein used to encapsulate the nucleic acid: the satellite viruses, which code for their own coat protein, and the satellite nucleic acids, which are packaged by the coat protein of the helper virus. The genetic material of satellite viruses and satellite nucleic acids comprises small molecules that have little, if any, sequence similarity to the helper virus genome. Replication of the satellite interferes to some degree with the replication of the helper virus and in some cases it affects the disease symptoms (Hull, 2002). Although there are numerous satellite RNAs associated with RNA plant viruses, as first reported in the 1960s (reviewed by Hu et al., 2009), it was not until 1997 that the first DNA satellite was described: ToLCV-sat, associated with *Tomato leaf curl virus* (ToLCV), a plant virus in the genus *Begomovirus* (family *Geminiviridae*) (Dry et al., 1997).

Members of this family have circular single-stranded DNA genomes encapsidated in twinned quasi-icosahedral (geminata) virions. Most geminiviruses have bipartite genomes and the two segments (referred to as DNA-A and DNA-B) are similar in size (2.5–3 kb). In contrast, monopartite begomoviruses have only one

genome component that is homologous to DNA-A. Geminivirus genomes are replicated by a rolling-circle mechanism initiated by the replication-associated protein (Rep), the only virus-encoded protein required for replication (Fontes et al., 1994; Stenger et al., 1991). The origin of replication is in a stem-loop structure located in the intergenic region (IR), which contains a conserved nonanucleotide sequence (TAATATTAC) (Orozco and Hanley-Bowdoin, 1996; Stanley, 1995). The IR also includes virus-specific iterated sequences upstream of the origin of replication to which Rep binds, referred to as iterons (Argüello-Astorga and Ruiz-Medrano, 2001; Argüello-Astorga et al., 1994).

The genus *Begomovirus*, which currently includes about 200 accepted virus species, is by far the largest of the four genera in the family *Geminiviridae*. Over the last 20 years or so, begomoviruses have emerged as serious constraints to the cultivation of a variety of crops in various parts of the world but especially in the tropics and subtropics (Morales, 2010; Navas-Castillo et al., 2011; Rojas and Gilbertson, 2008). Various devastating begomoviruses have also spread into more temperate regions, where they seriously reduce greenhouse crop production.

The ToLCV satellite (ToLCV-sat) is 682 nt in length and, with the exception of the hairpin structure that is conserved in all members of the family *Geminiviridae*, its sequence is unrelated to that of ToLCV, a begomovirus occurring in Australia. This satellite has no discernible effects on either viral replication or on the symptoms caused by ToLCV (Dry et al., 1997). Although it depends on the Rep protein of ToLCV for replication, the Rep binding motif of ToLCV-sat, located in a secondary stem-loop structure, does not appear to strictly fit the requirements that dictate the specificity of interaction of geminiviral

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Rep proteins with their cognate replication origin, which includes the iterons (Lin et al., 2003). In fact, ToLCV-sat replication is supported by a number of begomoviruses, including *Tomato yellow leaf curl virus* and *African cassava mosaic virus*, and even by the curtovirus *Beet curly top virus* (Dry et al., 1997).

To date, two additional types of DNA satellites associated with begomoviruses have been described: betasatellites and alphasatellites (Fig. 1). Betasatellites, first referred to as DNA- β , are satellite molecules that are associated with monopartite begomoviruses and are ~1360 nt in length (approximately half the size of the helper virus genome). Like ToLCV-sat, betasatellites are unrelated in sequence to their helper viruses and are dependent on them for replication, movement in plants and insect transmission. Betasatellites are widespread in the Old World, where the helper monopartite begomoviruses are known to occur, and although experimental transreplication by New World begomoviruses has been reported (Nawaz-ul-Rehman et al., 2009), they have not yet been found in the Americas. Betasatellites have a highly conserved organization consisting of an adenine-rich region (A-rich), a region that is conserved among all betasatellites (known as the satellite conserved region [SCR] and also present in ToLCV-sat), and a single open reading frame (ORF) in the complementary strand that codes for the β C1 protein (Briddon et al., 2003, 2008). The SCR contains a potential hairpin structure with the loop sequence TAA/GTATTAC, similar to that of the origin of replication of geminiviruses and nanoviruses (Briddon et al., 2003). Unlike ToLCV-sat, betasatellites augment the accumulation of their helper begomoviruses and enhance the symptoms induced in some host plants (Briddon et al., 2001; Nawaz-ul-Rehman and Fauquet, 2009; Patil and Fauquet, 2010; Saunders et al., 2000), probably due to the silencing suppressor activity of the β C1 protein (Cui et al., 2005; Saeed et al., 2005).

In addition to betasatellites, some begomovirus diseases are associated with an additional single-stranded DNA component, originally named DNA-1 and now known as alphasatellites (Briddon et al., 2004). Alphasatellites are approximately half the size of begomovirus components (~1375 nt) and show a common organization consisting of a single ORF coding for a Rep protein with similarity to those of nanoviruses (Mansoor et al., 1999; Saunders and Stanley, 1999), an

A-rich region and a predicted hairpin structure with the sequence TAGTATTAC (Briddon et al., 2004). Consequently, these molecules, unlike typical satellites, are capable of self-replication in host plants but require a helper begomovirus for movement within the plant as well as for insect transmission (Saunders and Stanley, 1999; Saunders et al., 2000). At least in one case, an unusual class of alphasatellites has been shown to attenuate begomovirus-betasatellite symptoms by reducing betasatellite DNA accumulation (Idris et al., 2011). Recently, two distinct alphasatellites have been found to be associated with New World begomoviruses. In Brazil, they were associated with two bipartite begomoviruses infecting weeds (*Euphorbia mosaic virus* and *Cleome leaf crumple virus*), and in both cases they contain the conserved genome features of alphasatellites, including a gene encoding a Rep protein, an A-rich region and a hairpin structure similar to those of a group of alphasatellites from Africa (Paprotka et al., 2010). The alphasatellite-like molecule found in Venezuela was associated with the bipartite begomovirus *Melon chlorotic mosaic virus*, and although its sequence diverged from that of typical Old World alphasatellites, it had all the aforementioned genome features of this type of DNA satellite (Romay et al., 2010).

In this work, we report the discovery and characterization of subviral molecules associated with bipartite begomoviruses from the New World which constitute a novel class of DNA satellites, in two malvaceous plant species. These molecules, in addition to sharing various genetic features with other DNA satellites associated with begomoviruses, contain nucleotide stretches of begomoviral origin, presumably the remains of recombination events involved in their origin.

Results and discussion

Identification of a small circular DNA molecule in *Malvastrum coromandelianum* infected with *Sida golden yellow vein virus-Malvastrum*

In a recent survey of begomoviruses infecting weeds in Cuba, we identified a new strain of the bipartite begomovirus *Sida golden yellow vein virus* (SiGYVV-Ma) infecting a plant (sample 177) of the malvaceous species *Malvastrum coromandelianum* (Fiallo-Olivé et al., 2012). Digestion with *Hind*III and *Nhe*I of DNA amplified by rolling circle amplification (RCA) from sample 177 rendered a high amount of linear product of ~700 bp, in contrast with the lower amount of begomoviral genome linearized with the appropriate enzymes (Fig. 2). We cloned the ~700 bp DNA fragments obtained after digestion with *Hind*III or *Nhe*I and obtained the complete sequence of five clones (two for *Hind*III and three for *Nhe*I). The cloned sequences were 687–689 nt in length (See Table 1 for GenBank accession numbers) and had a nucleotide identity of 99.3–100% between them. Based on comparison with known DNA viruses and satellites, these molecules appeared to be circular single-stranded DNA and contained several features of geminivirus origin, including a stem-loop with the conserved nonanucleotide TAATATTAC, a TATA box about 160 nt upstream from the stem-loop resembling the Rep gene promoter region, and iteron-like motifs located just upstream from the TATA box (Fig. 3A, C, D). The iteron-like sequences are associated, as shown for ToLCV-sat (Dry et al., 1997; Lin et al., 2003), with a stem-loop structure (Supplemental Fig. 1). They also contain an A-rich region, with three peaks of high A content, a hallmark of betasatellites and alphasatellites (Briddon et al., 2003, 2004) (Fig. 3B). In addition to the characteristics mentioned above, a nucleotide database search revealed a stretch of 23 nt (AGCCTTAGCTTCGCCGGAGCTGA) located between the TATA-box and the nonanucleotide that showed 95.7% identity with a region contained in the SCR of numerous betasatellites (Fig. 3C). Analysis of the coding regions indicated the presence of four small ORFs (38–53 aa) with no obvious RNA polymerase II promoter elements required to initiate

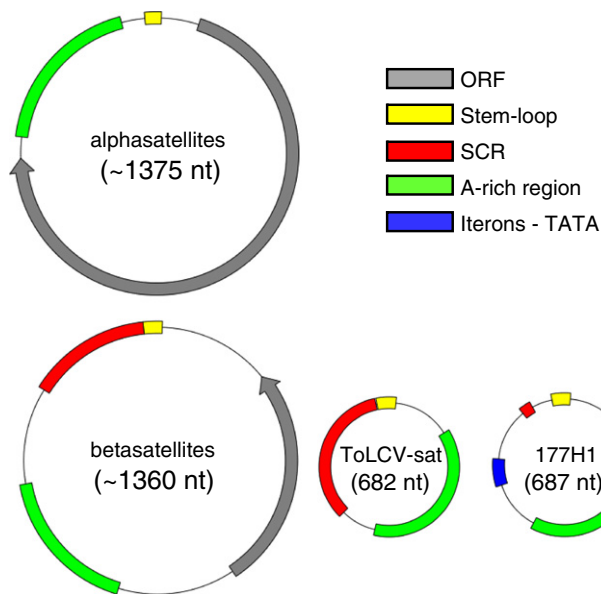


Fig. 1. Schematic representation of the DNA satellites previously known to be associated with begomoviruses (alphasatellites, betasatellites, ToLCV-sat), including the main genome features: ORFs, conserved stem-loop, satellite conserved region [SCR] and A-rich region. The clone 177H1 isolated from *M. coromandelianum* is included for comparison, with indication of the iteron-like region.

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