

Review

Role of Alphasatellite in Begomoviral Disease Complex

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Abstract. A circular single stranded satellite, called as alphasatellite (initially identified as DNA-1), was characterised and confirmed to be associated with the Geminivirus begomovirus-betasatellite complexes. Alphasatellites are single stranded DNA (ssDNA) components, frequently accompanying with monopartite begomovirus or some time with bipartite begomovirus and/or betasatellite complex. The genome of alphasatellite DNAs are nearly half size of its helper virus genome and have no similarity with it. Furthermore, their function in begomovirus-betasatellite complex is still unclear. Recent advances in application of molecular tools helped in finding new viruses and allied satellite components that further help in advancing our understanding of this satellite DNA and this evolution.

Keywords. Geminivirus, begomovirus, alphasatellite

Introduction

Geminiviruses are an emerging group of plant viruses infecting most of economically important crops and ornamental plants throughout the world (Mansoor *et al.*, 2003). Based on the host range, genome organisation and the vector, the Geminiviruses are classified into seven genera: *Becurtovirus*, *Eragrovirus*, *Turncutovirus*, *Topocovirus*, *Curtovirus*, *Mastrevirus* and *Begomovirus* (Adam *et al.*, 2013; Brown *et al.*, 2012). However, majority of the members of this family belongs to the genus *Begomovirus* and are spread by the whitefly, *Bemisia tabaci* (Bridson and Stanley, 2006). Viruses of this genus are distributed into two sub-groups; bipartite begomoviruses with DNA-A+DNA-B genomes and monopartite begomoviruses that have a single DNA chain homologous to the DNA-A of bipartite begomovirus. DNA-A component of bipartite and the single component of monopartite begomoviruses (homologues to the DNA-A) encodes all viral functions required for virus replication, control of gene expression and insect transmission. All begomoviruses have a potential stem-loop structure containing the nono-nucleotide sequence TAA/TATT/AC, necessary for replication.

In the last few years alphasatellite, the member of monopartite betasatellite/begomoviruses complexes, with a monomer of approximately 1375 nucleotide sequences, has attracted much attention and has become, probably, the most attentive scientific topic in the study of single stranded DNA (ssDNA) viruses. After the discovery of this satellite

in 1999, more than 150 alphasatellite sequences have been deposited in database to date, however, very little is known about their function(s) during begomovirus-satellites infections. Examples of stability and maintaining of the alphasatellite component in natural infection with several begomoviruses complex have been shown several times since its first discovery, but without gaining further insights on their function (Shahid *et al.*, 2014; Amrao *et al.*, 2010; Mubin *et al.*, 2010). Certainly, alphasatellites are non-essential for virus infection and appear to play no major role in the etiology of the infections with which they are associated (Mansoor *et al.*, 1999). However, recent reports showed that some alphasatellites can attenuate disease symptoms caused by begomovirus-betasatellite complexes in the early stages of infection (Idris *et al.*, 2011; Nawaz-ul-Rehman *et al.*, 2010). An overview of the origin and evolution of alphasatellites including the recent advances in understanding their molecular structure and their applications for reverse genetics are discussed.

General characteristics of alphasatellites. Despite that alphasatellites were discovered virtually 15 years ago, very little information is available up to now about its function(s). Alphasatellite molecules are mostly associated with monopartite begomovirus-betasatellite complex and also monopartite begomovirus can contain this component frequently (Shahid *et al.*, 2014; Harimalala *et al.*, 2013; Zhou, 2013; Zia-Ur-Rehman *et al.*, 2013; Mubin *et al.*, 2010; Dry *et al.*, 1997). On the contrary, a few bipartite begomoviruses have been reported to be associated with alphasatellite (Satya *et al.*, 2014; Paprotka *et al.*, 2010).

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Initially, alphasatellites were found in association with the begomovirus-betasatellite complex from the old world (OW). Nevertheless, some distinctive alphasatellites were recently discovered to be associated with the new world (NW) begomovirus complex (Fiallo-Olive *et al.*, 2012). Alphasatellites are believed to have evolved from satellite-like. Rep-encoding components associated with nanoviruses (Wyant *et al.*, 2012; Briddon and Stanley 2006; Saunders and Stanley, 1999), another family of plant ssDNA viruses. Alphasatellite was also found in association with a yellow vein disease in *Ageratum conyzoides* (weeds) (Saunders and Stanley, 1999).

Genome, genomic organization and replication mechanism. The size of alphasatellite is between 1,300 bp to 1,400 bp nucleotides in length and has three conserved domains: a hairpin structure, a rolling circle replication initiator protein (Rep) and a rich region (A-rich) (Fig. 1). The hairpin structure has a loop that includes a unique mono-nucleotide sequence, which usually varies from rest of the begomovirus components, TAG/TAT/TAC and differs from the TAA/TAT/TAC sequence of geminiviruses by one nucleotide (G instead of A on a third nucleotide). In both begomoviruses and nanoviruses this sequence contains the origin of replication (ori) and is nicked by the rolling circle replication initiator protein to initiate viral DNA replication. The Rep of alphasatellite is the only single large open reading frame in the virion-sense which is predicted to encode a 315 amino acid product similar to the replication associated protein of nanoviruses. An adenine-rich region

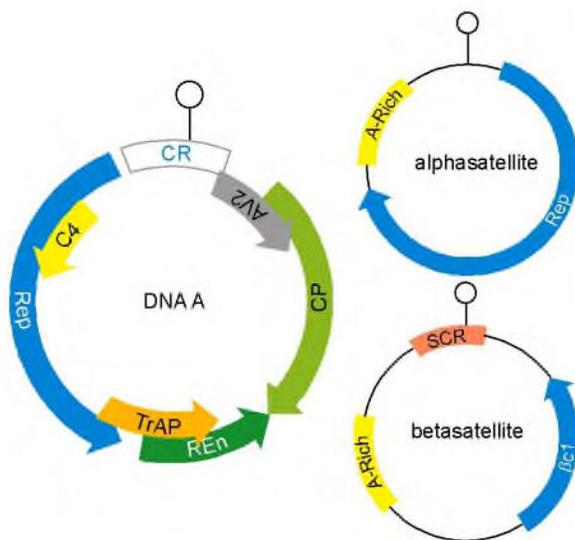


Fig. 1. Typical genome organisation of a monopartite-begomovirus satellite complex.

(approximately 200 bp with 45-52% adenine content) is also present, which is hypothesised to be a stuffer sequence that serves to fulfill the size constrain imposed by helper virus-mediated movement or encapsidation (Shahid *et al.*, 2014; Zhou, 2013). Alphasatellite can replicate autonomously and its replication is specifically mediated by its Rep (Tao *et al.*, 2004), while the replication of other components including betasatellite, is specifically mediated by the begomovirus Rep. This would suggest a difference in the begomovirus and alphasatellite replication origins. Recently, an alphasatellite associated with Okra leaf curl disease from West Africa (Kon *et al.*, 2009) is highly divergent molecule from previously characterized alphasatellites (Fiallo-Olive *et al.*, 2012; Saunders *et al.*, 2002) indicating geographically isolated evolution of a West African lineage of these satellites. The geographical distribution and the genetic diversity of these satellites are consistent with a long term association with monopartite begomoviruses (Briddon and Stanley, 2006).

Genetic variability. Most of the monopartite begomovirus-betasatellite complex associated with alphasatellites have been characterised in the OW. Previous studies have shown that cotton leaf curl Multan virus (CLCuMV), cotton leaf curl Burewala virus (CLCuBV), tobacco leaf curl Pusa virus (TbLCuPuV), Ageratum yellow vein virus (AYVV), tobacco curly shoot virus (TbCSV), tomato yellow leaf curl virus (TYLCV), East African cassava mosaic Kenya virus (EACMKnV) and mungbean yellow mosaic virus (MYMV) are usually associated with alphasatellite (Satya *et al.*, 2014; Shahid *et al.*, 2014; Harimalala *et al.*, 2013; Kumar *et al.*, 2011; Singh *et al.*, 2011; Mubin *et al.*, 2010; Xie *et al.*, 2010; Mansoor *et al.*, 1999) (Fig. 2). Recently, different alphasatellites such as cassava mosaic (virus) alphasatellite, *Gossypium darwinii* symptomless alphasatellite, Vernonia yellow vein Fijian alphasatellite associated with EACMKnV, and CLCuBV, MYMV were isolated from different hosts i.e., cassava, cucurbits and legumes (Satya *et al.*, 2014; Harimalala *et al.*, 2013; Zia-Ur-Rehman *et al.*, 2013). Interestingly, a strain of TYLCV originating from Oman has been shown to be associated with an unusual alphasatellite (Ageratum yellow vein Singapore alphasatellite), the only alphasatellite that was previously reported from Singapore back in 1999 (Idris *et al.*, 2011; Saunders and Stanley, 1999). Recently, Sida yellow vein China alphasatellite (SiYVCNA) has been identified in association with TYLCVV from main land Japan. However, the low levels of sequence divergence between all isolates of SiYVCNA suggests that this has only recently spread into Japan (Shahid *et al.*, 2014).

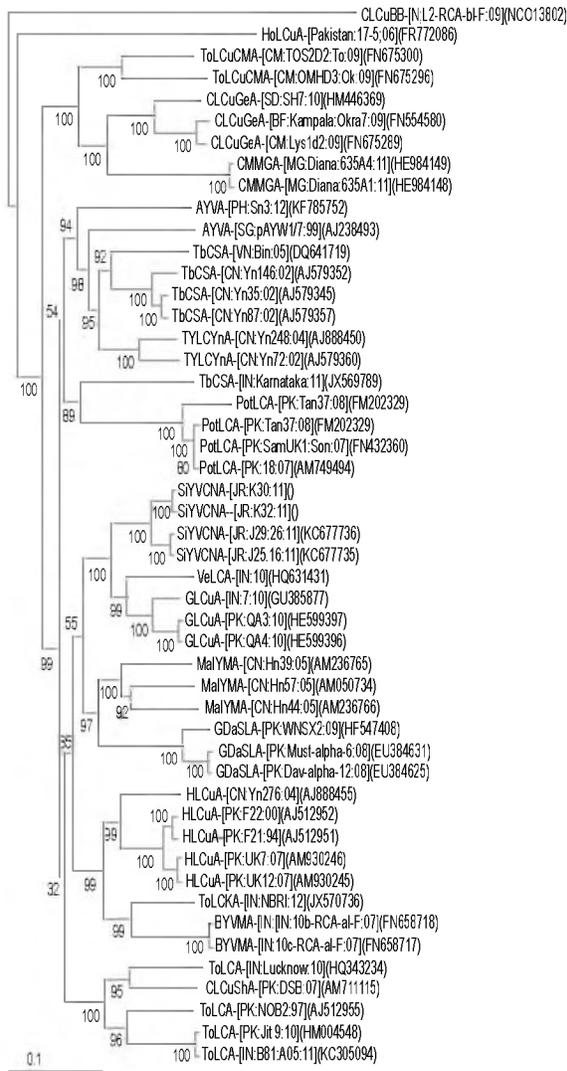


Fig. 2. Phylogenetic tree of commonly associated alphasatellites species.

Potential alphasatellite functions. Alphasatellites have no obvious contribution to symptoms induced by begomovirus-betasatellite disease complexes and appear to affect betasatellite replication but do not affect helper virus replication. However, some alphasatellites can attenuate disease symptoms caused by begomovirus-betasatellite complex in the early stages of infection. For example, Nawaz-ul-Rehman *et al.* (2010) have shown the alphasatellite Rep proteins encoded by two non-pathogenic alphasatellites, *Gossypium darwinii* symptomless alphasatellite (GDarSLA) and *Gossypium mustelinum* symptomless alphasatellite (GMusSLA). They can interact with Cotton leaf curl Rajasthan virus (CLCuRaV) Rep proteins (Table 1). Betasatellites depend solely for

replication on the Rep proteins encoded by their helper begomoviruses: binding between alphasatellite-Rep and helper virus Rep proteins may inhibit betasatellite replication and results in down regulated expression of β C1 and correspondent symptom amelioration. Also GDarSLA and GMusSLA alphasatellite-Reps have strong gene silencing suppressor activities (Nawaz-ul-Rehman *et al.*, 2010). Although further investigations are required to prove whether alphasatellite-Reps encoded by other alphasatellites also have silencing suppressor activities. Recently, alphasatellites have been found in association with bipartite begomoviruses in Venezuela and Brazil (Zia-Ur-Rehman *et al.*, 2013; Romay *et al.*, 2010), respectively. The DNA-2 type alphasatellite, a different alphasatellite (only two members) of this alphasatellite are found until now, one from *Ageratum* in Singapore and the other from tomato from Oman (Idris *et al.*, 2011; Saunders *et al.*, 2002). Although all these members contain conserved alphasatellite genome features, the DNA-2 type molecules are less homogeneous and have less than 50% nucleotide sequence identity with each other. The DNA-2 type alphasatellite identified in Oman can attenuate begomovirus symptoms and reduce accumulations of betasatellites (Idris *et al.*, 2011). Further studies are needed to confirm whether these satellite molecules are replicated by their helper virus (es) and whether they have role in pathogenesis similar to those of betasatellites and some alphasatellites. New technologies like vector-enabled metagenomics and the recent circular DNA genomics (Ng *et al.*, 2011) are anticipated to soon provide additional information about the field distributions of these novel satellites and their associated begomoviruses. The promising study about the function of this satellite indicate that the alphasatellite is most likely a molecular parasite of the helper begomovirus (Kon *et al.*, 2009).

Viral vectors based on alphasatellites. Many plant viruses have been adapted into expression and VIGS vectors for external protein expression (Gleba *et al.*, 2007) and silencing (Purkayastha and Dasgupta, 2009) of target genes in main crop plants. Recently, tobacco curly shoot alphasatellite (TbCSA) was successfully used to silence β -glucuronidase and the sulphur desaturase genes in different *Nicotiana tabacum* cultivars (Purkayastha and Dasgupta, 2009). Among that it can be used to investigate gene expression (or as an expression vector) on the entire host range of the begomoviruses/curtoviruses. Alphasatellite has some unique properties that make this component distinctive among other molecules. For example, it has Rep gene which makes the alphasatellite autonomous in replication, secondly it has a-rich region if deleted cannot effect on

Table 1. Alphasatellite associated with monopartite-betasatellite complex

Alphasatellite	Acc. no.	Associated Virus	Source
Ageratum yellow vein alphasatellite	AJ238493	<i>Ageratum yellow vein virus</i> (AYVV)	Saunders <i>et al.</i> , 1997
Ageratum yellow vein India alphasatellite	JX570736	<i>Tomato leaf curl Karnataka virus</i>	Chatchawankanphanich and Maxwell, 2002
Ageratum yellow vein Kenya alphasatellite	AJ512960	Diversity of alphasatellite	Briddon <i>et al.</i> , 2004
Ageratum yellow vein Pakistan alphasatellite	FR772085	<i>Cotton leaf curl Burewala virus</i>	Iqbal <i>et al.</i> , 2013
Ageratum yellow vein Singapore alphasatellite	AJ416153	AYVV	Saunders, 1999
Cassava mosaic Madagascar alphasatellite	HE984148	<i>East African cassava mosaic Kenya virus</i>	Harimalala <i>et al.</i> , 2013
Cleome leaf crumple alphasatellite	FN436007	<i>Cleome leaf crumple virus</i>	Paprotka <i>et al.</i> , 2010
Cotton leaf curl Dabwali alphasatellite	AJ512957	Diversity of alphasatellite	Briddon <i>et al.</i> , 2004
Cotton leaf curl Gezira alphasatellite	FM164740	AYVV	Leke <i>et al.</i> , 2013
Croton yellow vein mosaic alphasatellite	FN658711	<i>Croton yellow vein mosaic virus</i>	Zaffalon <i>et al.</i> , 2011
Euphorbia yellow mosaic alphasatellite	FN436008	<i>Euphorbia yellow mosaic virus</i>	Fernanda <i>et al.</i> , 2011
Gossypium darwinii symptomless alphasatellite	EU384606	<i>Cotton leaf curl Rajasthan virus</i>	Nawazul-Rehman <i>et al.</i> , 2010
Hibiscus leaf curl alphasatellite	AJ512950	Diversity of alphasatellite	Briddon <i>et al.</i> , 2004
Hollyhock yellow vein symptomless alphasatellite	FR772086	<i>Hollyhock yellow vein virus</i>	Saunders <i>et al.</i> , 2000
Lantana yellow vein alphasatellite	KC206075	<i>Lantana yellow vein virus</i>	Marwal <i>et al.</i> , 2013a
Malvastrum yellow mosaic alphasatellite	AM050734	<i>Malvastrum yellow mosaic virus</i>	Guo <i>et al.</i> , 2006
Malvastrum yellow mosaic Cameroon alphasatellite	FN675297	<i>Tomato yellow leaf curl China virus</i> (ToLCCNV)	Leke <i>et al.</i> , 2011
Melon chlorotic mosaic virus alphasatellite	FM163578	<i>Melon chlorotic leaf curl virus</i>	Romay <i>et al.</i> , 2010
Mesta yellow vein mosaic alphasatellite	JX183090	<i>Mesta yellow vein mosaic virus</i>	Chatterjee <i>et al.</i> , 2005
Okra leaf curl alphasatellite	AJ512954	Diversity of alphasatellite	Briddon <i>et al.</i> , 2004
Sida yellow vein Vietnam alphasatellite	DQ641718	<i>Sida yellow vein Vietnam virus</i>	Ha <i>et al.</i> , 2006
Tobacco curly shoot alphasatellite	AJ579361	<i>Tomato yellow leaf curl China virus</i> (ToLCCNV)	Xie <i>et al.</i> , 2010
Tomato yellow leaf curl China alphasatellite	AJ579358	ToLCCNV	Xie <i>et al.</i> , 2010
Verbesina encelioides leaf curl alphasatellite	HQ631431	<i>Hollyhock yellow vein virus</i>	Prajapat <i>et al.</i> , 2011
Vernonia yellow vein Fujian alphasatellite	JF733780	<i>Vernonia yellow vein Fujian virus</i>	Zulfikar <i>et al.</i> , 2012
Vinca yellow vein alphasatellite	KC206076	<i>Vinca yellow vein virus</i>	Marwal <i>et al.</i> , 2013b

its replication, lastly this molecule is quiet small and easy to manipulate. Shahid *et al.* (2009) have shown by agro-inoculation studies with a-rich deleted cotton leaf curl Multan alphasatellite (CLCuMA) that this sequence is not required for the infectivity or maintenance of CLCuMA. Also CLCuMA has a wider host range and can successfully be maintained by a large number of diverse *Begomovirus* species. The ability to amplify itself is useful in a vector since it will increase the copy number (and thus also expression) of inserted sequences. deletion of a-rich region to increase the insert size and wider host range makes it a potentially useful vector (Tao and Zhou, 2004). The a-rich deleted CLCuMA was maintained in plants in the presence of a begomovirus. Although little is yet known about the maintenance of alphasatellites by begomoviruses, it is likely that high-level replication of these molecules is required for their maintenance, which depends upon its own Rep. There is no evidence for a (strong) selection mechanism for maintenance of alphasatellites. Maintenance of alphasatellites can simply be a selection of numbers; plants containing high levels of the satellite allow cell-to-cell movement by the virus encoded movement proteins or infection to the next plant by the vector of the helper

begomovirus. Tao and Zhou (2004) used modified CLCuMA for virus induced gene silencing vector in plants. The same vector was used to successfully silence the chelates gene (ChII). One of the advantages of an alphasatellite vector, over many of the other vectors, is that it can, at least in theory, be used with different *Begomovirus* or even *Curtovirus* (Saunders *et al.* 2002).

Recent research advances. Recent progresses in research comprises of the construction of the alphasatellite-based vectors, the development of the first VIGS system for different agricultural crops, the description of new alphasatellites, improvement in diagnostics, and new information on the begomovirus-satellite complex.

Conclusion

The role that alphasatellites play in begomovirus-satellite disease complex is still generally unidentified. The recent advancement and emerging potential of Next Generation Sequencing approaches will undoubtedly contribute considerably to the elucidation of the aetiology of many of these alphasatellite associated diseases. The fairly recent discovery of alphasatellite in Japan (Shahid *et al.*, 2014)

and its presence in papaya gardens in Nepal (Shahid *et al.*, 2013) suggest that its occurrence and possible role in disease in other agricultural-producing regions need to be investigated. What effect the presence of an alphasatellite and the defective allied component may have on future begomovirus-betasatellite complex is not clear.

Whereas outdated research focused on the detection and characterisation of prevailing and new begomovirus-satellite complexes, we believe research on (i) the elucidation of the etiology of these disease complexes (ii) the development of resistance using non-transgenic approaches, and (iii) studies on the molecular interaction of alphasatellites and their helper viruses with their original host. As efficient tools are being developed now, future research with begomoviruses, as well as with all other whitefly-vectoring viruses, has to move from typical (model) plants like *Nicotiana benthamiana* towards other host plants to allow the study of symptomology, pathogenicity, host-plant response and viral determinants of vector transmission in their natural host.

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