

Genome organization of satellite DNA beta molecule associated with Mungbean yellow mosaic India virus Gujarat, India

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ABSTRACT

Recent advancements in genomics have unveiled fascinating insights into the intricate world of plant pathogens. The discovery of an intriguing association between the DNA beta and MYMIV-[IN:Ana: CpMBKA25:04], a strain containing 1367 base pairs of nucleotides, has sparked a wave of scientific curiosity. Through sequence identity matrix analysis, it was revealed that this strain shares a striking 96% similarity with the Potato apical leaf curl virus, closely followed by the Tomato leaf curl beta satellite virus [Accession No. AY230138] at 95.3%. In contrast, its relationship with the beta satellite Bhendi yellow vein beta satellite [Accession No. AJ308425] stood at a comparative 45.7% when compared against seventeen other DNA beta isolates infecting various crops. Noteworthy features of the beta satellite sequences include a distinctive putative stem-loop structure TAATATT/AC in the common region and a recurring GCTACGC repeat sequence. An A-rich region spanning from nucleotides 910 to 1120 further adds to the genetic complexity of this novel strain. Intriguingly, an analysis of the amino acid sequence unveiled additional residues V, C, and N at the carboxyl end of the β C1 protein, setting it apart from its counterparts. The identity matrix analysis established a 95.9% similarity between β C1 and the beta satellite associated with Tomato leaf curl Karnataka virus and Potato apical leaf curl virus, phylogenetic analysis placed the beta satellite linked with MYMIV-[IN:Ana: CpMBKA25:04] in a distinct category within the Papaya leaf curl virus family, shedding light on its unique evolutionary lineage and biological significance in the realm of plant virology.

Keywords: An A-rich region spanning from nucleotides 910 to 1120 further adds to the genetic complexity of this novel strain.

INTRODUCTION

Begomoviruses are known to be a great threat to several agriculture crops. They include cotton, okra, tobacco, and tomato and yellow mosaic diseases in grain legumes in the tropics for a long time. Since the Green Revolution (GR) period, begomoviruses spread their host range enormously and spreading to weeds also [1] [2]. Grain legumes cultivated as rainfed and irrigated crops in Gujarat are severely affected by whitefly-transmitted yellow mosaic disease (YMD) which is a bipartite begomovirus replicating in a rolling circle mechanism [3] belonging to the family *Geminiviridae*. Monopartite and begomoviruses are associated with a small satellite designated as DNA- β . Uptil now more than 200 satellite sequences have

been established from variety of plants which includes a range of weeds, vegetables and fibre crops in Africa and Asia, reflecting their diversity and impact on agriculture throughout the Old World [4]. Basil Kassanis in 1962 used the term satellite virus for the 17 nm diameter particles associated with tobacco necrosis virus. There are three types of circular ssDNA satellite molecules. They are alphasatellites, betasatellites, and deltasatellites are associated with diseases caused by begomoviruses. The satellites, in general, may have originated from genetic elements that were once part of the viral genome but eventually lost their ability to replicate independently [1] and also replicate similar rolling circle and recombination-dependent replication mechanisms [5]. As there is no report on the molecular characterization of beta satellite viruses causing YMD in legumes in Gujarat, the present study is focused on molecular cloning of genomic components of yellow mosaic viruses (YMV) infecting legumes and establishment of their relationship with other reported beta satellite viruses.

MATERIALS AND METHODS

MYMIV infected legume samples were used for DNA using the modified CTAB method [6]. PCR amplification was done based on the sequence of DNA beta satellite region using universal primers [7] PCR products were cloned in pGEM-T Easy vector. Computer analysis of sequence was carried out using the software programme Bioedit version 5.0.9, CLASTALW, the neighbour-joining method by MEGA version 3.1.

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Table 1: List of beta satellites associated with begomoviruses with their accession numbers from GenBank used for all sequence analysis

Sr. No.	Name of Virus	Accession No
1	Mungbean yellow mosaic India virus associated DNA beta:IN:Ana: CpMBKA25:04	DQ118862
2	Tomato leaf curl virus-associated DNA beta	AY230138
3	Tomato leaf curl virus-associated DNA beta	AY438557
4	Tomato leaf curl virus-associated DNA beta	HM101175
5	Potato apical leaf curl virus associated DNA beta	EF043234
6	Papaya leaf curl virus associated DNA beta	AY244706
7	Cotton leaf curl Multan virus associated DNA beta	EU384579
8	Cotton leaf curl virus-associated DNA beta	AY083590
9	Bhendi yellow vein mosaic virus-associated DNA beta virus	AJ308425
10	Tomato leaf curl-associated DNA beta	AJ316035
11	Ageratum yellow vein virus-associated DNA beta	EF527824
12	Chilli leaf curl virus satellite DNA beta C1 gene	FN179279
13	Tobacco leaf curl virus-associated DNA beta	NC_013800
14	Zinnia leaf curl virus-associated DNA beta C1 gene	AJ542499
15	Tomato leaf curl Karnataka virus-associated DNA beta	AY754813
16	Tomato leaf curl Bangalore virus-[Ban5] satellite DNA beta	EU280314
17	Cowpea severe leaf curl-associated DNA beta	AY728263

Results and Discussion

The sequence analysis of the beta satellite of Mungbean yellow mosaic India virus-[IN:Ana: CpMBKA25:041350] showed 1367 base pairs of nucleotides and their genome analysis is shown in Fig. 1-4 and Table 2. The sequence analysis showed the presence of iteron-like elements homologous which help in the replication. TAATATTAC is also present in the β satellite which is a common feature of geminiviruses. The Gujarat isolate showed the presence of an adenine-rich region, a satellite-conserved region and a single ORF encoding β C1 protein. Several studies show that the symptom development depends upon β C1 protein. The present study supports the presence of β satellite which is occurring mainly in Africa and Asia with maximum diversity from the Indian continent. The similarity identity matrix showed that the whole genome had 96 % similarity with Potato apical leaf curl virus-associated DNA beta and approximately 96 per cent identity with Potato apical leaf curl virus-associated DNA beta and Tomato leaf curl virus associated DNA beta viruses (Karnataka isolate) in β C1 region. The amino acid sequence showed similarity with those of other viruses which consisted of extra amino acid residues V, C, N in the β C1 protein at the carboxyl end. When all the isolates were compared with MYMIV-[IN:Ana: CpMBKA25:04] isolates it is found to be a distinct isolate belonging to the Papaya leaf curl virus. Begomoviruses family Geminiviridae causes viral diseases in economically important crop plants. Genomically begomoviruses possess two genomic components DNA A and DNA B. DNA β components do not show any homology to helper

begomoviruses. But they depend upon them for replication, encapsidation and movement. DNA β play a role in the host-specific symptoms in plants. Previous studies show that DNA β components exist in nature. So present paper also reveals the presence of DNA β component present in the legumes. Usually, β satellites are found to be associated with monopartite begomoviruses. Nowadays they are found to be spreading in bipartite begomoviruses spreading in the old world too. Earlier it has been reported from MYMIV infecting cowpea [8], mungbean [9] and tomato [10], chilli [11] [12] and kenaf [13]. Other part of the world showed the association of DNA β in Sri Lankan cassava mosaic virus [14], Tomato yellow leaf curl Thailand virus [15], and the New World bipartite Cabbage leaf curl begomovirus [16]. The presence of β satellite associated with begomoviruses was found to play a significant role in the evolution of begomovirus. Initiation of replication as well as the control of complementary-sense gene expression in DNA β is initiated by TAATATTAC [17]. TAATATTAC is a universally conserved region within the loop of a hairpin structure and it is a nonanucleotide sequence. Rep protein nicks at its downstream of the virion sense strand [18] [19]. Tobacco ring spot nepovirus was identified as the first satellite RNA in 1969 [20]. The first monopartite begomovirus satellite ToLCV-sat was isolated from tomato plants [21]. Different studies and its compilation shows that DNA satellites usually consists of 1350 nucleotides and they are called as DNA β . They are usually seen in the case of monopartite begomoviruses [7] [22] [23] [24] [25]. Betasatellites are usually the half of the genome size of the helper begomoviruses [26].

Satellite virus they depend upon the helper virus for its replication. Ageratum yellow vein virus (AYVV) infects ageratum plants when inoculated with the virus. But when it is inoculated in *Nicotiana benthamiana*, it produces asymptomatic infection and indicates the requirement of the presence of a helper virus like satellite molecule [27]. Cotton leaf curl Multan virus (CLCuMV) and Tomato leaf curl virus (ToLCV) showed severe symptoms in the plants in the presence of the helper virus [28] [29] [30]. This shows the role of β satellites in the disease symptoms [31], virus movement in plants [32], binds DNA in a sequence non-specific manner [33], is a suppressor of RNA silencing [34] [35], forms homo-multimeric complexes *in planta* [35], inter feres with host gene expression [36] and has been shown to interact with a variety of host factors [37] [38]. So similarly at present too MYMIV infects legumes and its ability to infect *Nicotiana benthamiana* is yet to be explored with its presence of β satellite virus. In the present studies, components contain an A-rich region suggesting that they may have originated as a bonafide component of another pathogenic agent before being captured by the begomovirus necessitating a slight increase in size as shown by earlier studies [4]. In addition to the above mentioned A-rich region, β satellite virus components usually in common poses a single gene β C1. It is a highly conserved sequence consisting of 80 nucleotides which is known as the satellite-conserved region (SCR) and PCR primers based on this sequence have provided a simple and robust method for the isolation of DNA- β components [23]. During the past years, it has been found from the database that more than one hundred full-length DNA- β sequences have been deposited in from plant species belonging to the family *Malvaceae* and those associated with non-*Malvaceae* [39]. A similar trend also can be seen in the present study. The present study also shows its phylogenetic relationship showing as a distinct clade differing from the isolates of monopartite viruses like Tomato leaf curl virus and the *Malvaceae* family. Eralier studies showed that distinct betasatellite species share a high level of sequence identity with respect to β C1 gene which are responsible for inducing distinct symptoms. The whitefly performance is also dependenet on the serine-33, a conserved phosphorylation site in betasatellite-encoded β C1 protein which suppresses the plant terpenoid-based defense[40]. The identification of a relatively high level of diversity of beta satellites in okra showed possibility of development of resistance by both conventional breeding and transgenic approaches [26]. Previous studies interpret the possibility of monopartite begomoviruses of the other host might move legumes aided by the associated betasatellites. Still a lot of research is yet required to know association of betasatellites with MYMIV and MYMV will affect its host range, virulence and transmission to contain the YMD spread and mitigate the yield loss [41].



Fig. 2 Itron acharacteristics in the common region of beta satellite of Mungbean yellow mosaic India virus-[IN:Ana: CpMBKA25:04].

complement(189..557)

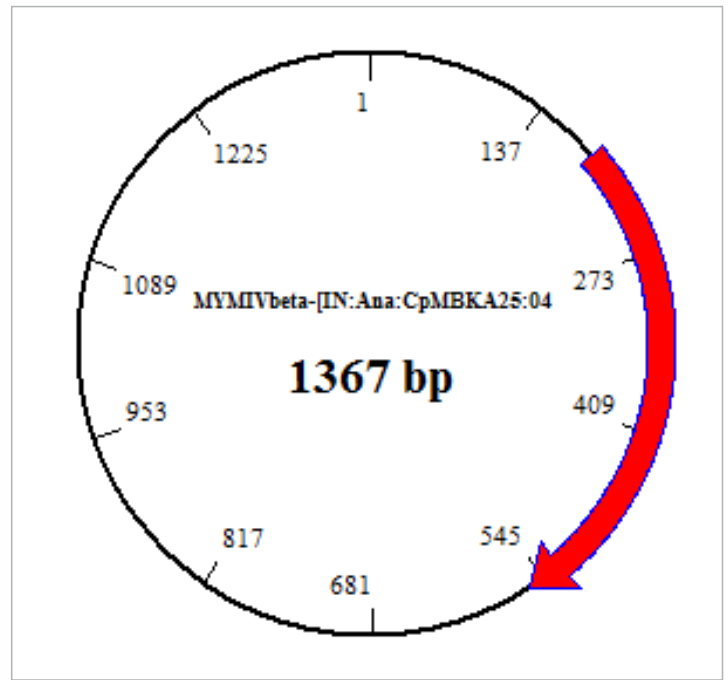


Fig 1 Genome organization of beta satellite of Mungbean yellow mosaic India virus-[IN:Ana: CpMBKA25:04]

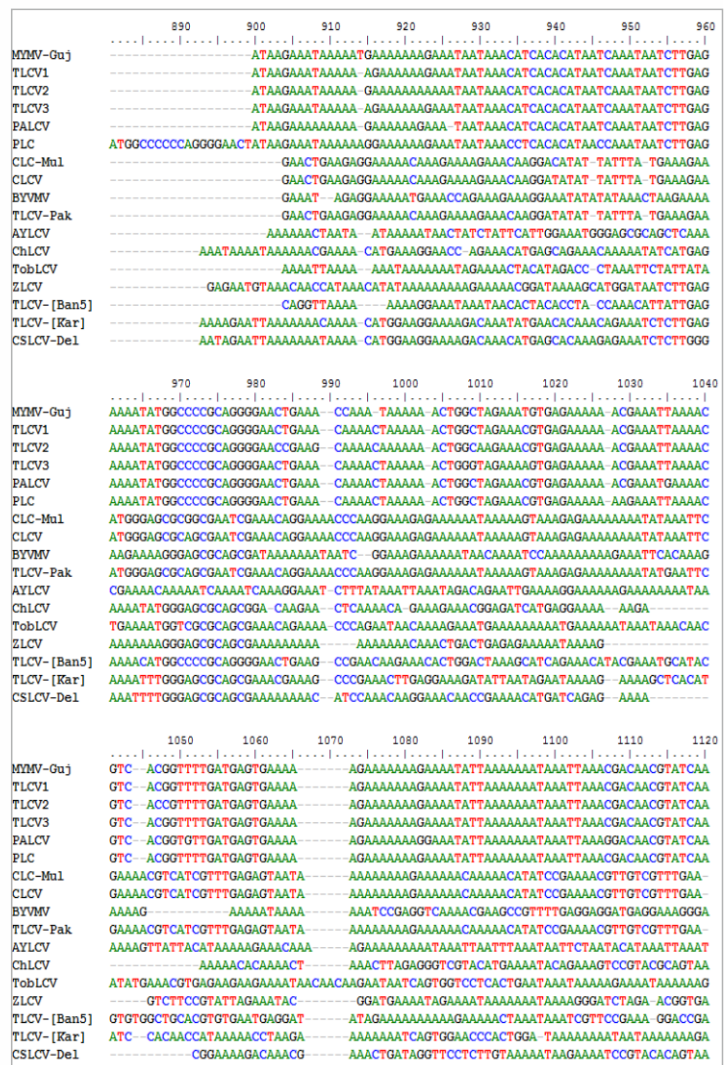


Fig. 3A – rich region in the genome of the beta satellite of Mungbean yellow mosaic India virus-[IN:Ana: CpMBKA25:04]

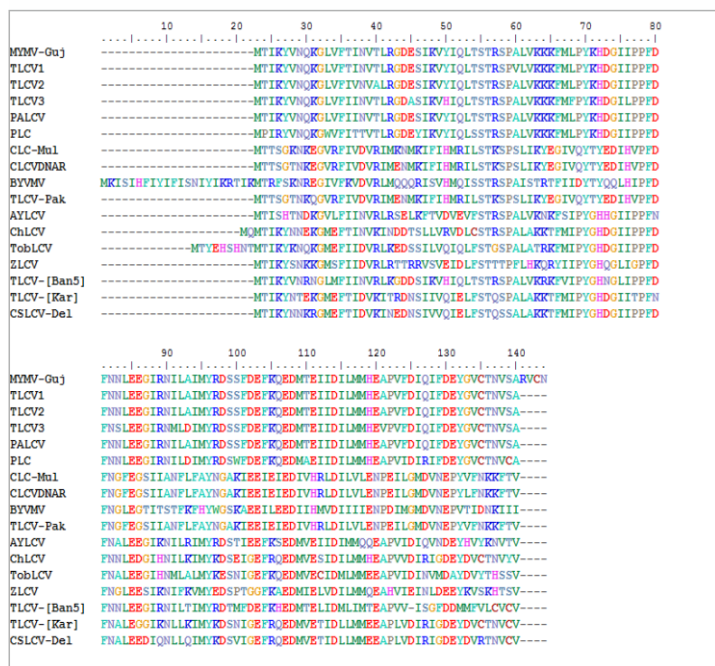


Fig 4 Predicted Amino acid sequence of the beta satellite of Mungbean yellow mosaic India virus-[IN:Ana: CpMBKA25:04].

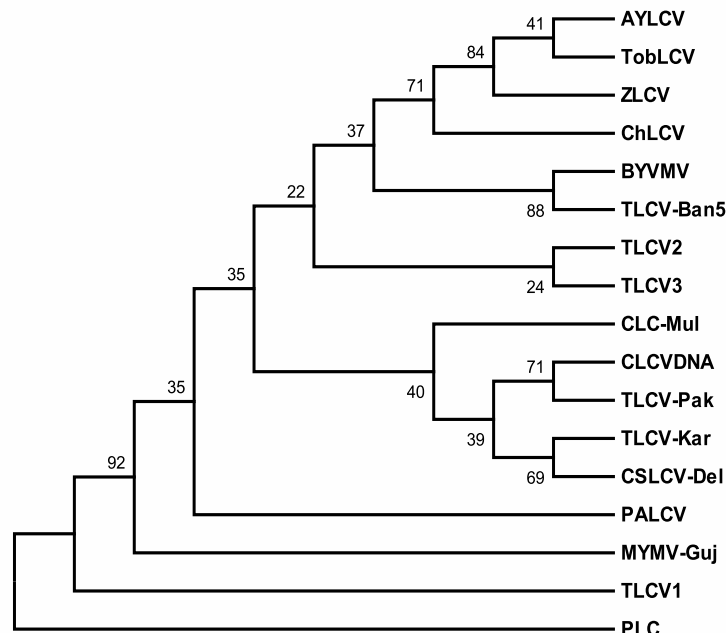


Fig:5 Phylogenetic relationship between sequence of the beta satellite of Mungbean yellow mosaic India virus-[IN:Ana: CpMBKA25:04] with other β DNA of begomoviruses

Table2: Percentage identity of the nucleotide sequence in the complete genome and β c1 of the beta satellite of Mungbean yellow mosaic India virus-[IN:Ana: CpMBKA25:04] with other β DNA of begomoviruses

β DNA of begomovirus isolates	The whole genome of the Beta satellite	Beta C1
Tomato leaf curl virus-associated DNA beta	92.0	95.9
Tomato leaf curl virus-associated DNA beta	95.3	94.2
Tomato leaf curl virus-associated DNA beta	94.3	89.3
Potato apical leaf curl virus associated DNA beta	96.0	95.9
Papaya leaf curl virus associated DNA beta	80.8	85.2
Cotton leaf curl Multan virus associated DNA beta	48.1	24.5
Cotton leaf curl virus-associated DNA beta	50.4	24.5
Bhendi yellow vein mosaic virus-associated DNA beta virus	45.7	23.6
Tomato leaf curl-associated DNA beta	50.1	24.5
Ageratum yellow vein virus associated DNA beta	55.6	60.6
Chilli leaf curl virus satellite DNA beta C1 gene	61.5	66.9
Tobacco leaf curl virus-associated DNA beta	61.1	58.7
Zinnia leaf curl virus-associated DNA beta C1 gene	56.7	49.1
Tomato leaf curl Karnataka virus-associated DNA beta	73.3	68.0
Tomato leaf curl Bangalore virus-[Ban5] satellite DNA beta	59.8	61.4
Cowpea severe leaf curl-associated DNA beta	61.2	61.4

CONCLUSION

Satellite DNA beta found to be associated with Mungbean yellow mosaic India virus-[IN:Ana: CpMBKA25:04]. Present study shows the presence of DNA-β components that pose a threat to agriculture throughout the Old World. The recent isolation of novel satellite components suggests that other disease complexes and subviral components may exist and its role in symptom and transmission requires much exploration. DNA-β and DNA-A and DNA-B can show the possibility of interacting with different begomoviruses.

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