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Research article

A NEW VARIANT OF *CASSAVA MOSIAC VIRUS* CAUSES MULBERRY MOSAIC DISEASE IN INDIA

A.V. Mary Josepha Shery

Central Sericultural Research and Training Institute, Central Silk Board, Srirampura, Mysore-570 008

ABSTRACT: Mulberry with typical symptoms characteristic to *Gemini virus viz.*, enations, mosaic, deformed leaves, curling and stunted growth was collected from Thiruvananthapuram district, Kerala, India. The disease is transmitted through mechanical transmission from the sap of the infected plant to disease free mulberry seedlings and indicator plants, *Nicotiana tabaccum* and *N. benthamiana*, confirming the presence of a sap transmissible pathogen. The cassava biotype of *Bemisia tabaci* transmitted the disease with an AAP 24h and IAP 6h. Amplification for the Gemini virus specific primers was obtained from the total DNA isolated from the infected plants. Further with the primers specific for the coat protein gene (cp, AV1 gene of the A genome) and movement protein gene (mp, BC1gene of the B genome) of the ICMV the ~810*bp* and ~ 860*bp* specific amplifications were obtained from the Thiruvananthapuram isolate and the plants inoculated with the sap of Thiruvanathapuram isolate and from plants on which vector transmission done using *B. tabaci* cassava biotype.The amplified samples were sequenced and submitted the nucleotide sequences to NCBI data base, accession code for cp nucleotide sequence is FJ.827040.2 and that for mp nucleotide sequence is HM.138689.1. The two nucleotide sequences showed 98-99% identities and closest relationship with *Indian Cassava Mosaic Virus* (ICMV) and *Sri Lankan Cassava Mosaic Virus* (SLCMV). *Bemisia tabaci* is confirmed as the vector of the disease by transmission studies and obtained amplification of 810 *bp* with cp primer from the *B. tabaci* adults of cassava biotype used for transmission studies.
Key words: *Bemisia tabaci*, *Morus alba*, mulberry mosaic disease, *Mulberry mosaic virus*

*Corresponding author: A.V. Mary Josepha Shery, Central Sericultural Research and Training Institute, Central Silk Board, Srirampura, Mysore, India, E-mail: maryjosephasherry@gmail.com

INTRODUCTION

Mulberry (*Morus alba*) is the food plant of the monophagous silkworm, *Bombyx mori* L. Victory1 (V1) is one of the most popular varieties of *M. alba* in South India. In this variety mosaic symptoms were noticed from Kerala, India. V1 variety developed mosaic symptom in addition to enations, curling and chlorosis and 2% disease occurrence was noticed in the mulberry gardens in different locations under study. In Kerala Cassava, *Manihot esculenta*, plants are found infected with Cassava Mosaic Disease (CMD). Both *Indian Cassava Mosaic Virus* (ICMV) and *Sri Lankan Cassava Mosaic Virus* (SLCMV) were characterized from CMD infected cassava plants from different locations of India. They belongs to the family *Geminiviridae* and genus Begomovirus. A recent report using RFLP techniques [1] has already suggested that the majority of cassava-infecting begomoviruses in Southern India resemble SLCMV rather than ICMV. Studies of Rothenstein *et al.* [2] by analyzing full length DNA sequences and detailed phylogenetic study proposed that the new virus isolates are variants which may have originated by recombination between parental viruses resembling SLCMV and ICMV. By the proximity of the cassava cultivations near the infected mulberry gardens and infestation of *Bemisia tabaci* on the mulberry plants, possibility of transmission of the CMD to mulberry plants was suspected.

The Symptoms of typical *Gemini virus* infection were expressed on the B. *tabaci* infested mulberry plants. Earlier reports on the natural host range of *Cassava Mosaic Gemini Viruses* (CMGs) were that CMGs are largely restricted to cassava and a few of its wild relatives such as *Manihot glaziovii* [3]. However a number of reports are available later on the occurrence of CMGs on other host plants supporting the theory of its origin from indigenous virus attacking wild plants [4, 5, 6]. Transmission of *Indian Cassava Mosaic Virus* (ICMV) from cassava to cucumber was reported by Menon and Raychaudhuri [7]. The association of ICMV with Yellow Mosaic Disease of bitter gourd (*Momordica charantia*) has been detected in Tamil Nadu, India [8]. Complete genome of the *Jatropha Mosaic Virus* earlier reported as one similar to ICMV elsewhere [9] by analyzing the partial sequence, was confirmed as an isolate of ICMV [10]. In the present study the disease transmission studies using both sap inoculation and vector transmission methods were done using mulberry seedlings and indicator plants. The molecular characterization of the mosaic virus isolated from mosaic disease affected mulberry plants and indicator plants were done to identify the virus.

MATERIALS AND METHODS

A Survey was conducted in Thiruvananthapuram district of Kerala. Infected plants collected were kept in a glass house in insect free conditions and was used for further experiments and labeled as Thiruvananthapuram isolate (Fig.1). The sap transmission was done from the Thiruvananthapuram isolate on *Nicotiana tabacum* var. Delcrest and *N. benthamiana* (Fig.2) and seedlings of mulberry varieties *viz.*, *Morus multicaulis*, K2 and S36. From the symptom expressed plants, back inoculation was done on healthy seedlings of mulberry and *Nicotiana* Spp. grown in insect free conditions. Infected leaves (0.1gm) were ground in two dilutions *viz.*, 5 and 10 ml of Sorensen's buffer / phosphate buffer (pH 7.2). Four to five leaf stage seedlings of mulberry (K2, S36 and *Morus multicaulis*), 4-5 leaf stage seedlings of *N. tabacum* and *N. benthamiana* and 2-3 leaf stage cassava seedlings still having cotyledons were used for both sap inoculation and vector transmission studies. Plants were raised in sterilized potting mixture and kept in insect free cages. The plants were kept in the dark for 4-6 hours before inoculation. Abrasive used to be carborandum powder (600 mesh) and stabilizers were sodium diethyldithiocarbamate (DIECA), 2-mercaptoethanol (2-ME) and polyvinyl pyrrolidone (PVP). Stabilizers like 2-ME and PVP were always used together and DIECA was used alone. Five minutes after inoculation plants were washed using distilled water and kept in insect free cages and symptom expression was observed daily. Each experiment done on 3-5 plants and repeated thrice.

The *B. tabaci* pure culture was maintained on seedlings of cassava raised from seeds sown in sterilized potting mixture. These plants were maintained in whitefly free cage of 70 cm (height) by 42 cm by 42 cm covered with organdy cloth on three sides. Transparent plastic sheets covered one side and top of each cage and they were kept in a glass house. Red-eye nymphs of *B. tabaci* collected from the healthy mulberry plants maintained in glass house were kept in Petri plates and after emergence they were released to the virus free cassava seedlings maintained in the cages. The Leaves of the mulberry seedlings will become coarse in the caged conditions, whereas cassava seedlings are easy to handle and suitable for raising in laboratory conditions, hence they are used for culturing of the *B. tabaci.* Besides that, mulberry is the host of two different species of *B. tabaci* reported to infest cassava and other plants respectively. Culturing the whiteflies collected from mulberry plants on cassava seedlings will help in isolating the different species and developing a monoculture of the cassava biotype due to its host specificity. Seedlings were replaced periodically after introducing new insect free seedlings into the cage for the insect to shift from the old ones. The maintained culture completed 20 generations in the cage. The time given is supposed to be enough for the establishment of a host specific *B. tabaci* mono culture in the respective host plant. The molecular characterization of the *B. tabaci* in the pure culture was done and confirmed as cassava biotype using mtCO1 gene analysis.

The red eye nymphs of *B. tabaci* collected from the pure culture described above, were kept on moderately wet filter paper in Petri plates of 20cm diameter. Female flies emerged from these were transferred into clip cages of 5 cm diameter and kept on the infected leaves Thiruvananthapuram isolate for 24h for virus acquisition and then on virus free plants of mulberry, *N. tabacum and N. benthamiana* for inoculation. The time given for IAP (Inoculation access period) were 6h, 12h, 24h, to 2-7days. After the experiment the insects were removed and the plants were disinfected with pesticides.

Tender leaves of the symptom expressed mechanically inoculated samples, vector transmitted plants and the Thiruvananthapuram isolate were used for DNA isolation. Total DNA was extracted [11]. Two grams fresh tissue was ground in a sterile mortar with a pestle in 20ml of preheated (65°C) extraction buffer (100mM Tris, pH 8.0; 50mM EDTA; 500mM NaCl; and 10mM 2-mercaptoethanol with a pinch of PVP) immediately after grinding, added 400µl of 5% SDS to this and kept for 1h incubation at 65°C.

After taking it out from the water bath added 5ml of 5M potassium acetate and incubated at 4°C for 30min. The tube was centrifuged at 12,000 rpm for 5 min at 4°C. The debris free supernatant fluid was removed to a new tube and the DNA was precipitated by adding 10ml of isopropanol. It was mixed well and kept in 1h incubation at -20°C. Afterwards the tube was centrifuged at 10,000 rpm for 20min at 4°C. To the pellet added 1ml of sterile distilled water and kept for overnight incubation at 4°C. To this added 8µl of RNase and incubated at 37°C. Extracted it once with chloroform-isoamyl alcohol. The tube was centrifuged at 10,000rpm for 15 min at 4°C. The aqueous layer removed and DNA precipitated using 200µl of 3M sodium acetate and 2ml of ice cold 100% ethanol and by gentle inversion the solution mixed and incubated at -20°C for 1h. The Incubated solution centrifuged at 10,000 rpm for 15 min at 4°C. The pellet washed in 70% ethanol thrice and air dried pellets dissolved in 500µl of sterile distilled water. DNA isolated from the whiteflies used for transmission studies according to De Barro and Driver [12] in which for homogenization 10µl of lysis buffer (50mM KCl, 10mM Tris pH 8.4, 0.45% Tween 20, 0.2% gelatine, 0.45% NP40, 60µg/ml proteinase K) were used**.** Polymerase chain reaction (PCR) was carried out in 25 µl reaction mixture having 2.5 µl of 10 X Taq buffer A (containing 15mM $MgCl_2$), 1 µl (2.5mM) dNTP, I µl each primers, 4µl of 22µg / ml DNA and 0.5 µl (3U/ µl) Taq polymerase obtained from B'lore Genei. Reactions were carried out in MJ-Research PTC-200 thermo cycler. The programme used was initial denaturation for 2min at 94ºC followed by 30 cycles with cyclic denaturation at 94°C for 1min, annealing 55°C for 2min and an extension for 3min at 72°C with a final extension for 5 min at 72°C. The products were separated by electrophoresis on 1% agarose gel stained with 1% ethidium bromide. Amplification of the virus was done by polymerase chain reaction (PCR) using Rojas primer PALIc1960 and PARIv722 [13]. After confirming the presence of Gemini virus, primers specific for the coat protein of the ICMV, ("5'GGATCCATGTCGAAGCGACCA3' - 5'AAGCTTTTAATTGCTGACCGA3'"[14] were used in PCR and got amplification of ~810 bp and an amplification of ~ 860bp for movement protein gene got from the same plant with the primer 5'ATGGAGAATAATAGTAGCAA3' - 5'TTATACATTTTTGGATACAT3' [14]. Amplified products were visualized under transilluminator (Genei UVitec) and documented through the Gel Doc system (Alpha imager, Alpha Innotech, USA). Negative controls were PCR mix without DNA sample. Molecular marker used was a 100 bp ladder of B'lore Genei Pvt. Ltd. PCR products were separated on 1% agarose gel and excised from the gel and purified using a gel extraction kit (Qiagen, GmbH, D-40724, and Hilden, Germany). The eluates were used for sequencing. Sequenced the DNA with dideoxy sequencing in the automated sequence facility of B'lore Genei. Sequences were edited using the BioEdit software 7.0 [15]. The nucleotide sequences were compared with those in the NCBI database using the Basic Local Alignment Search Tool (BLAST, http://www.ncbi.nlm.nih,gov/BLAST). The sequences obtained were aligned using sequences from NCBI which showed similarity to them using Clustal W multiple alignment programme of BioEdit software. The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model [16]. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. Estimates of Evolutionary Divergence between Sequences were conducted using the Tajima-Nei model [17]. The number of base substitutions per site from between sequences is shown. Evolutionary analyses were conducted in MEGA6 [18].

RESULTS

Both Sorensen's buffer and phosphate buffer gave positive results, in sap inoculation. But symptom expression was earlier by 1-2 days when Sorensen's buffer was used. Stabilizers DIECA and PVP with 2-ME gave symptom expression in dilutions of both 5ml and 10ml. More than 50% of the mechanically inoculated plants developed symptoms after 9-25 days. The mulberry plants developed symptoms like puckering, netting, chlorosis, vein clearing and epinasty. The tests satisfy the Koch's postulates that a virus is a causative agent of disease by developing symptoms in indicator hosts, *N. tabacum* and *N. benthamiana* and successful back inoculation to mulberry. In indicator plants local lesions developed within 2-5 days and symptoms developed within 20-25 days. *N. benthamiana* developed netting, curling and cupping symptoms whereas *N. tabacum* developed cupping, curling, irregular shaped leaves and tip burning.

A single fly with 24h AAP and 6h IAP was able to transmit the disease from mulberry to mulberry. In the vector transmitted mulberry plants symptoms developed after 10-15 days. Puckering, netting and epinasty were the common symptoms in all the vector transmitted mulberry plants, only K2 seedlings developed mosaic symptom after vector transmission. Symptom development occurred after 18 days in it. Varieties S36 and *M. multicaulis* developed netting, curling and cupping. In *N. tabacum and N. benthamiana* yellow netting observed in vector transmitted plants. The vector transmission was 100% in mulberry, *N. tabacum* and *N. benthamiana.* Even though the initial symptom expressed were different in vector transmitted mulberry seedlings, after 2 months all the plants had similar symptoms like slender creepy stems, bunching of buds and puckering and epinasty in leaves.

PCR and sequencing

As expected bands of ~1280 was consistently amplified by PALIc1960 and PARIv722. Amplification of ~810 bp for AV1 gene and~ 860bp for BC1 gene were amplified from Thiruvananthapuram isolates, sap inoculated and vector transmitted plants. The sequences obtained were submitted to the NCBI Gen Bank (National Center for Biotechnology Information) and the accession numbers assigned to nucleotide sequence for cp gene is FJ.827040.2 and that for mp gene is HM.138689.1. Amplification of cp gene also obtained from the *B. tabaci* adult fly used for the transmission studies and from the mechanically inoculated *N. tabacum.* In phylogenetic tree the mp nucleotide sequence had maximum closeness (99%) to the nucleotide sequence of ICMV Kollihills isolate DQ017669.1, SLCMV-Ker4 AJ575821.1, SLCMV Ker20 AJ579308.1, and SLCMVAM238431.1. and they formed a separate clad. The CP gene showed maximum similarity to following accessions and formed a clad, ICMV AY998122.1, ICMVDQ780004.1, ICMVAY769966.1, ICMVAF 423180.1 and ICMV AY 312989.1. The nucleotide sequence obtained from the sap inoculated *N. tabacum* showed maximum identity (98%) to ICMV Kolli hillsAY998122.1 ICMV(Tri) isolate AF423180.1 and SLCMV Ker 15AJ890228.1.

Figure-1: Tender leaf of mulberry, Thiruvananthapuram isolate, showing typical Gemini virus symptom

Figure-2: Picture of indicator plant, *Nicotiana benthamiana,* which developed yellow netting after mechanically inoculated with sap of Thiruvanathapuram isolate.

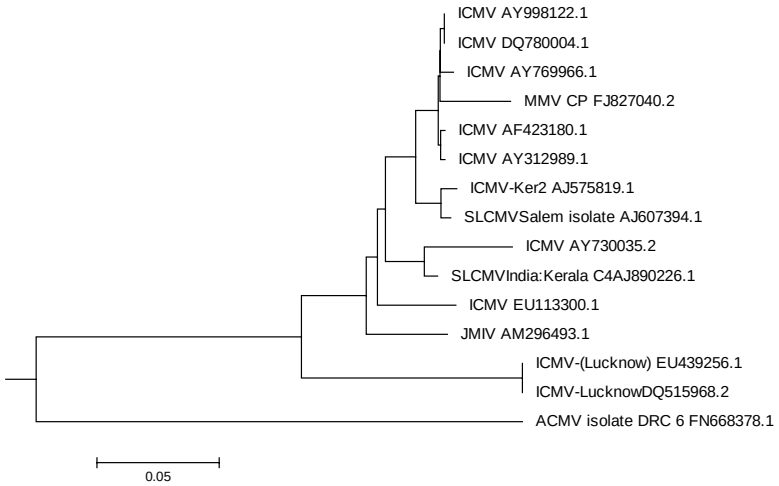

Figure. 3: Relationship dendrogram of the coat protein (AV1 gene) nucleotide of mulberry mosaic virus with other whitefly transmitted geminiviruses (WTGs)

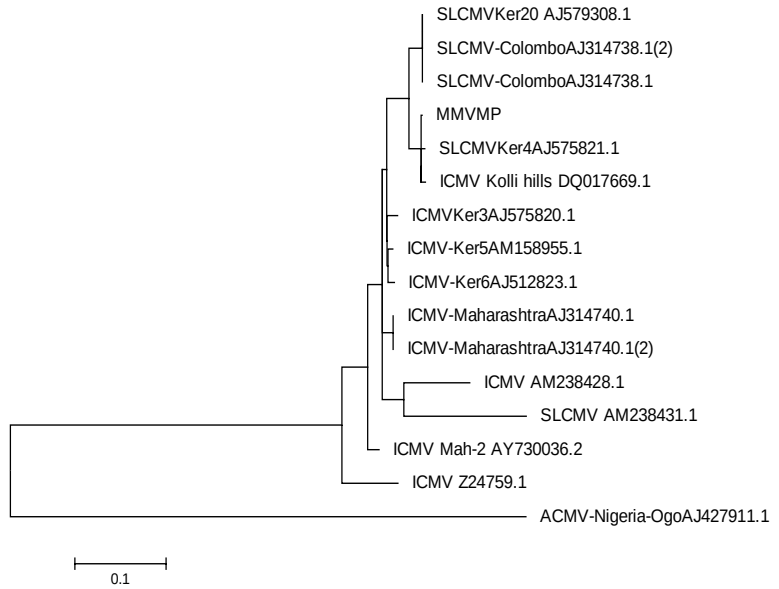

Figure.4: Relationship dendrogram of the movement protein (BC1gene) nucleotide of mulberry mosaic virus with other whitefly transmitted geminiviruses (WTGs)

Table.1. Estimates of Evolutionary Divergence between Sequences used for phylogenetic analysis of AV1 gene of Mulberry Mosaic Virus

ICMVAF423180.1														
ICMVAY312989.1	0.003													
ICMVAY998122.1	0.005	0.005												
ICMVDQ780004.1	0.005	0.005	0.000											
ICMVEU113300.1	0.059	0.059	0.061	0.061										
ICMVAY730035.2	0.078	0.078	0.081	0.081	0.082									
ICMVAY769966.1	0.009	0.009	0.007	0.007	0.065	0.085								
JMIVAM296493.1	0.065	0.065	0.067	0.067	0.072	0.088	0.067							
ICMVLucknowEU439256.1	0.156	0.156	0.153	0.153	0.160	0.176	0.158	0.165						
ICMVLucknowDQ515968.2	0.156	0.156	0.153	0.153	0.160	0.176	0.158	0.165	0.000					
ICMVKer2AJ575819.1	0.031	0.031	0.029	0.029	0.058	0.084	0.032	0.073	0.144	0.144				
SLCMVSalemisolateAJ607394.1	0.027	0.027	0.025	0.025	0.062	0.088	0.029	0.077	0.149	0.149	0.011			
SLCMVIndia:KeralaC4AJ890226.1	0.042	0.042	0.044	0.044	0.070	0.041	0.047	0.064	0.149	0.149	0.047	0.051		
ACMVisolateDRC6FN668378.1	0.342	0.342	0.340	0.340	0.333	0.344	0.351	0.328	0.370	0.370	0.331	0.340	0.342	
MMVCP	0.032	0.032	0.030	0.030	0.090	0.102	0.034	0.090	0.180	0.180	0.057	0.053	0.068	0.359

Table 2. Estimates of Evolutionary Divergence between Sequences used for phylogenetic analysis of BC1 gene of Mulberry Mosaic Virus

ICMVMaharashtraAJ314740.1													
SLCMVColomboAJ314738.1	0.027												
SLCMVKer20AJ579308.1	0.027	0.000											
SLCMVKer4AJ575821.1	0.022	0.035	0.035										
ICMVKollihillsDQ017669.1	0.020	0.036	0.036	0.008									
ICMVMah2AY730036.2	0.009	0.023	0.023	0.019	0.020								
ICMVKer5AM158955.1	0.018	0.026	0.026	0.022	0.023	0.014							
ICMVKer6AJ512823.1	0.016	0.025	0.025	0.020	0.022	0.012	0.009						
ICMVKer3AJ575820.1	0.022	0.027	0.027	0.026	0.027	0.018	0.018	0.016					
ICMVZ24759.1	0.027	0.036	0.036	0.029	0.030	0.023	0.023	0.022	0.027				
ICMVAM238428.1	0.128	0.131	0.131	0.128	0.129	0.123	0.118	0.122	0.106	0.134			
SLCMVAM238431.1	0.170	0.187	0.187	0.158	0.165	0.169	0.172	0.172	0.175	0.181	0.196		
ACMVNigeriaOgoAJ427911.1	0.751	0.752	0.752	0.756	0.743	0.765	0.765	0.752	0.768	0.770	0.802	0.888	
MMVMP	0.019	0.032	0.032	0.003	0.005	0.016	0.019	0.018	0.023	0.026	0.124	0.160	0.752

Table.3. Symptoms developed after sap transmission.

Host plant	Inoculum	Symptoms	Day of observation
Mulberry	Mulberry	LL	3- 5
		PU, CR, N and EN	9-25
N. benthamiana	Mulberry	LL	2-5
		YN and *CR*	20-25
N.tabaccum	Mulberry	LL	2-5
		CR, CU, IR, BLTB	20-25
Mulberry	*N. benthamiana* Mechanically inoculated with mulberry	Local lesions	2
		Plants died	7

BLTB- bud and leaf tip burning, CU-cupping, CR-curling, EN-Epinasty, N-Netting, PU-Puckering, YN-yellow netting, LL-Local lesions, IR- Irregular shaped leaf

DISCUSSION

The mulberry mosaic disease is transmitted by sap inoculation to mulberry, *N. tabacum* and *N. benthamiana* and by back inoculation from *N. tabacum and N. benthamiana* to mulberry. The vector of the disease is identified as the cassava biotype of *B. tabaci.*. Reports are available on mechanical inoculation of ICMV to 48 species of Solanaceae and Euphorbiaceae and SLCMV to 39 species of Solanaceae[19,20]. Berrie *et al.*[21] reported the successful inoculation of SACMV to *Phaseolus vulgaris*, *Malva parviflora* and *Manihot esculenta* and development of symptoms like leaf curling, chlorosis and stunting. Gao *et al.*[10] succeeded in agro infiltration of the *Jatropha mosaic virus* into *N. benthamiana*. ICMV reported from bitter gourd was not transmitted through sap from bitter gourd to bitter gourd, but the virus was positively transmitted by sap to *N. benthamiana* or through whitefly [8].

In vector transmission a single fly with 24h AAP can infect the mulberry plant with 6h IAP. Morris *et al.* [22] gave AAP of 48h and an IAP of 74 h for transmission. AAP for 5 - 7.5 min and 1, 2, 3, 5, 10, 24, 48,74h were tried by Antony *et al.*[23] to study the AAP time requirement for the transmission of ICMV and got amplification in all. The bands were thick from 3 h onwards, which indicates the presence of more inoculum. With 24h AAP and 48h IAP 25 flies were required to transmit *Cotton leaf curl Multan virus*-Hib (Ban4) (CLCuMV–Hib (Ban)) from *Hibiscus* to *Hibiscus* with 20% result [24], the transmission increased to 70% when 50 flies were used. Following viruses required much shorter time periods, *Cotton Leaf Curl Virus* needed 1h AAP and 5min IAP [25] and *Tomato leaf curl Bangalore Virus* (Ban4) (ToLCBV-(Ban4) required 10min AAP and 20min IAP [26] and single *B. tabaci* transmitted *Cotton leaf curl Cochran virus* (CLCuV-K) [25], ToLCBV-(Ban4) [26] and *Potato yellow mosaic virus* (PYMV) [27]. Moreover the above three begomoviruses required only 5-15 viruliferous *B. tabaci* to achieve 100% transmission efficiency compared to the 50 whiteflies required for achieving only 70% transmission by CLCuMV-Hib(Ban). This suggests that differences occur in the vector transmission abilities in begomoviruses occurring in South India. The AAP differs in different biotypes and the latent period for CMV (*Cassava Mosaic Virus*) is reported as 35 weeks between inoculation and symptom expression [28]. But in the ICMV transmission studies of Antony *et al.* [29] using cassava biotype of *B. tabaci*, typical CMD (Cassava Mosaic Disease) symptoms of foliar chlorotic mosaic or mottle and distortion of leaves developed within nine days.

The reported ICMV from bitter gourd shows ninety nine percentage similarity to the ICMV kolli hills isolate with accession number AY998122.1[8]. The complete DNA-A nucleotide sequence of ICMV-Dha is most similar to those of ICMV-Ker (IN:Ker2) (92.4%), ICMV-IN (IN:Mah) (90.9%) and ICMV-IN (IN:Mah2) (90.1%)[10]. The *Jatropha mosaic virus* from India shows 94.3% and 94.7% similarity to ICMV[9, 30]. In the present study two complete genes, that of movement protein and coat protein were isolated from Thiruvananthapuram isolate. These two genes from DNA A and DNA B respectively support the bipartite nature of the *Mulberry mosaic virus.*The CP gene is the most highly conserved gene in the family Geminiviridae. CP sequence, which effectively predicts discrete strains, species, and taxonomic lineages of begomoviruses and has been accepted by the ICTV (International Committee on Taxonomy of Viruses) as a desirable marker for virus identity when a full-length genomic sequence is not available as it has optimally averages variable and conserved regions more in line with the variation in the whole genome[31,32, 33, 34,35,36]. Exchange of coat protein genes, between transmissible and nontransmissible viruses and between viruses of different genera, has strongly implicated the coat protein as the major protein involved in determining vector specificity as well as mediating the interactions involved in the transmission process [37, 38]. The identification of the MMV with the two available ORFs, cp and mp is substantial. The close nucleotide identity of the MMV with ICMV and SLCMV in the genotypic analysis leads to the conclusion that it is an ICMV variant like the ICMV-Dha virus isolated from Jatropha [10]. The ICTV geminiviridae study group proposed new species demarcation criteria, the most important of which being an 89% identity threshold between complete DNA-A component nucleotide sequences of begomoviruses. Brown *et al*.[36] used the core cp region for predicting the identities of the closest begomoviruses relative and other members of the same sub cluster, and often the probable identification of a particular virus or strain were possible with it. Fauquet and Stanley [39] identified two peaks in the pair wise comparison distribution below the taxonomic level of species, one at 90-91% identity that may correspond to strains and one at 96-98% identity that may correspond to variants. In the present study all the three sequences obtained were similar to ICMV and SLCMV isolates and the similarity was above 96% and hence the MMV can be considered as a variant of ICMV and SLCMV. Fauquet and Fargette [3] opined that CMBs are largely restricted to cassava and a few of its wild relatives such as *Manihot glaziovii* and *Jatropha multifida* (Euphorbiaceae) but it was suspected to infect *Hewittia sublobata* (Convolvulaceae) in Kenya [4] and *Laportea aestuans* (Urticaceae) in Nigeria [6]. Berrie *et al*. [21] succeeded in inoculating *Agrobacterium tumefaciens* inoculated with *South African cassava mosaic virus* (SACMV) to plant species, *Phaseolus vulgaris, Malva parviflora* and cassava and they displayed following symptoms like leaf curling, chlorosis and stunting. Alabi *et al.* [41] and Mgbechi-Ezeri [42] isolated *African cassava mosaic virus* (ACMV) from weed plants, *Senna occidentalis, Leucana leucocephala* and *Glycine max* from leguminosae family, *Ricinus communis, Manihot glaziovii from euphorbiaceae and Combretum confertum* from Combretaceae with 96-98% nucleotide sequence identity and isolated *East African cassava mosaic virus*(EACMV) from four of the above species except *Ricinus communis* with 96-99% similarity with corresponding cassava species from West Africa, giving a definitive evidence for the natural occurrence of ACMV and EACMCV*(East African cassava mosaic Cameroon virus*) in plant species besides cassava. Occurrence of ACMV and EACMCV on plant species other than cassava provides definitive evidence for its natural occurrence of it in other plant species. Hence the concept of a restricted host ranges of CMGs ruled out. Since cassava was introduced from South America in the 16th Century, it is likely that CMBs endemic to Africa, infecting indigenous African plant species have become adapted to cassava upon its introduction[43]. This same explanation can be adopted in the case of ICMV and SLCMV from India. It is interesting to note that all of the natural hosts of ACMV and EACMV documented so far in Nigeria occur in the Humid Forest and Derived/Coastal Savannah agro ecological zones of the country. These two zones have been documented as hot spots for CMD with high whitefly population and thus support its weed origin [44]. Studies conducted in Uganda also have shown that the whitefly vector could colonize different plant species besides cassava [45]. A wide host range of CMBs and whitefly vectors could facilitate their survival during periods when cassava is not available. Mulberry reported as a host for overwintering for both *B. tabaci* and its parasitoids near cotton plantation and acted as a reservoir for both when cotton plants are not available [46]. Mulberry is cultivated in large scale in India for sericulture and the perennial plant can act as a reservoir for the virus and its vector. The spread of ICMV once believed to be restricted to Indian subcontinent is a threat as it will result in the formation of new recombinants and synergistic effect with other CMBs. Similar situations can occur in India too as ACMV was reported from certain pockets of Kerala, India [20].

CONCLUSION

From this study a bipartite begomovirus causes MMD and it was transmitted by *B. tabaci.* The virus is a new variant of ICMV. Detection of CMVs in hosts other than cassava may result in the development of more virulent recombinants. Tamil Nadu has both mulberry and cassava cultivation in large scale and the presence of ICMV on mulberry must be taken seriously as it can cause epidemics as occurred in Africa. The first molecular identification of a begomovirus in India that is closely related to *Indian and Sri Lankan cassava mosaic virus* that causes a mosaic disease on mulberry is reported in this paper.

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