Molecular characterization and Phylogenetic Analysis of Citrus *Mosaic Badna Virus* (CMBV) Associated with Sathgudi sweet orange

BY

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Citrus is grown in 140 countries of the world and is one of the choicest fruit having high consumer's preference both as fruit as well as its refreshing processed juice.

Introduction

Citrus

Family : Rutaceae

Tribe : Citreae

Sub Tribe: Citrineae

Genus: Citrus

Area in India: 0.56 mha

Production in India: 4.58 mt

Productivity in India: 12-13 tons/ha

(The low productivity of citrus in India is due to many abiotic and biotic stresses.)

World Productivity: 28-30 tons/ha.

Important members : Sweet Orange, Acid Lime, Rangpur Lime, Pummelo, Rough Lemon, Mandarin and Grape Fruit

Major Citrus growing areas in India

Important diseases: Citrus tristeza, Indian Citrus Ringspot, Citrus mosaic virus, Citrus Greening,Citrus exocortis, Citrus Gummosis, Citrus canker etc.

Citrus mosaic virus **disease caused by Citrus mosaic Badnavirus (CMBV), is an important disease in citrus growing areas particularly southern part of India.**

Importance of work: for determine variability in the viral genome.

First report in India -

Dakshinamurti and Reddy, 1975

– Sathgudi Sweet orange - Andhra Pradesh

Ahlawat, 1985

– Khasi Mandarin – N. E.India

Disease Incidence

10 to 70% **(Ahlawat *et al.*, 1996)**

77% reduction in Fruit yield

10% reduction in Juice and ascorbic acid content

(Reddy G.S *et al.*, 1985)

CMBV

Ahlawat et al.,1996 did partial characterization of citrus mosaic virus (CMBV) which they name as citrus yellow mosaic badnavirus. However, as per 8th edition of ICTV, The virus has been named as *citrus mosaic virus*

(According to Huang and Hartung,2001)

- Family: Caulimoviridae
- Genus: Badnavirus
- Non enveloped bacilliform virus
- 30 X 120-150nm
- Circular ds DNA
- 7559 bp.
- six ORFs
- Pararetrovirus
- Huang and Hartung,2001, First confirm the full genome of citrus mosaic badnavirus in sathgudi sweet orange

Molecular characterization of *Citrus mosaic virus*

Symptoms induced by *Citrus mosaic virus* on sathgudi sweet orange under field condition.

Method Materials:

Collection of culture: Survey of sathgudi sweet orange (*Citrus sinensis* (L.) Osb) was done in the Nagri village of Chittor, district which is about 40 km away from Tirupati in Andhra Pradesh during November 2006. Two orchards of sathgudi sweet orange which was having approximately 440 plants were surveyed for presence of mosaic and greening disease symptoms. Symptomatic leaves from sathgudi sweet orange plants showing mosaic symptoms were collected and stored at -80 0C.

Graft transmission and establishment of culture: Bud sticks from plants showing mosaic symptoms were wedge grafted on 10 plants each of 1 year old healthy seedlings of sweet orange (*Citrus sinensis* (L) Osbeck and acid lime (*Citrus aurantifolia* (Christm). The grafted and healthy plants were maintained in the insect proof glasshouse. The grafted plants were observed for about 6 months and plants showing symptoms were used for further studies.

Electron microscopy of CMBV: The leaf-dip preparation was used for detection of CMBV and stained with 20% uranyl acetate examined under transmission electron microscope (JEOLJEM-1011) at Plant virology unit, Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi 110012.

Isolation of total DNA from plant leaves

1.The total genomic DNA was isolated from healthy and CMBV infected leaf from glass house using DNeasy plant mini kit method (Qiagen Gmbh, Hilden, Germany). The method is described below.

2.100 mg of diseased and healthy leaf tissue for CMBV were taken separately for total DNA isolation. The samples were washed and ground to powder with the help of pestle and mortar in liquid nitrogen.The powdered samples were immediately transferred to autoclaved 1.5 ml eppendorf tubes.

3.To this 400 µl of AP1 buffer and 4µl of RNase A (100mg/ml) were added and vortexed vigorously for proper mixing. The mixtures were incubated for 10 min at 650C. 4.The tubes were inverted 2-3 times during incubation period.After 10 mins 130 µl of AP2 buffer was added to lysates, properly mixed and kept in ice for 5 mins. Lysates were centrifuged at 12,000 rpm for 5 mins.

5.The supernatant lysates were transferred into the QIAshredder spin columns (lilac colour) placed in 2 ml collection tube and centrifuged at 12,000 rpm for 2 mins.

6.The flow through were transferred to a new eppendorf tubes without disturbing cell debris.To the flow through 1.5 volumes (of flow through) of AP3/E buffer was added and mixed properly by pipetting.

7.The 650 µl of mixtures were transferred into the DNeasy columns placed in a 2 ml collection tubes. 8.Centrifuged for 1 min at ≥ 8000 rpm and flow through was discarded. Step 8 was repeated for remaining mixture, flow through and collection tubes were discarded.

9.The DNeasy mini spin columns were placed in a new 2 ml collection tubes. 10. 500 µl of AW buffer was added to columns and centrifuged for 1 min at ≥8000 rpm.11. Flow through was discarded and columns with collection tubes were reused in step 11.

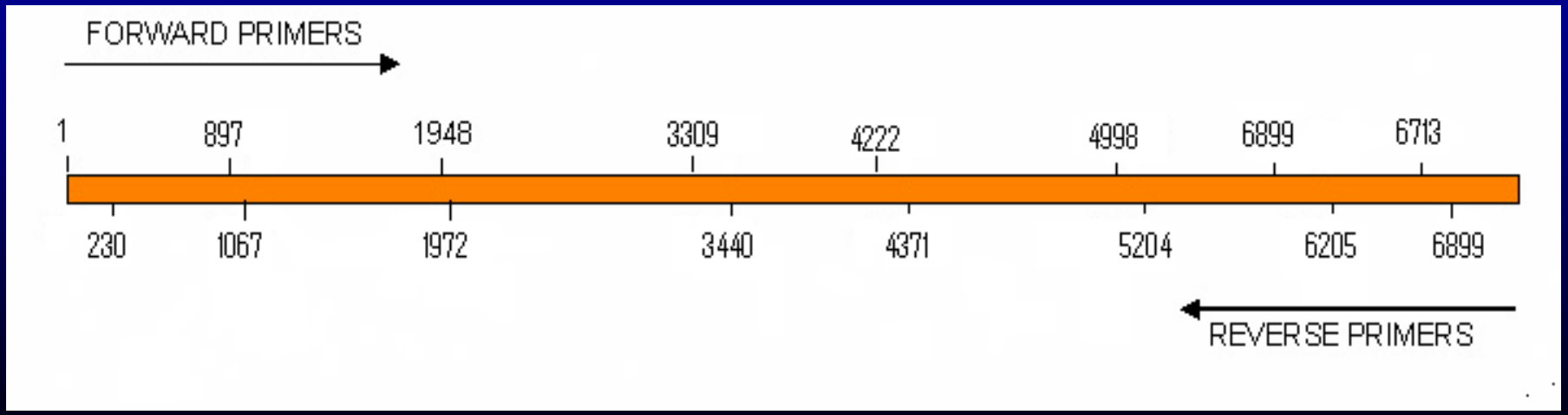
12.500 µl of AW buffer was added to columns and centrifuged for 2 min at maximum speed to dry the membrane. 13.The DNeasy columns were transferred to a new 2 ml microcentrifuge tubes and 75 µl of preheated (650C) AE buffer was added directly into DNeasy column membrane.

14.Columns were incubated at room temperature and centrifuged for 1 min at ≥8000 rpm to elute DNA from the membrane of the columns.15. 75 µl of collected DNA was stored at -20oC for further use.

Primers for CMBV genome amplification

Eight sets of overlapping primers (17-22 nucleotides) were designed manually for full length genome amplification of citrus mosaic virus (CMBV) associated with sweet orange, Nagri isolate (CMBVSON). The full length genome sequence of CMBV available in GenBank (accession number AF3476695) was used for designing and synthesis of primers. The primers were synthesized at Biobasic, Canada.

Sweet orange – Andhra Pradesh (CMBVSOH) Accession Number : AF3476695



Details of primer sets used for PCR amplification of full length genome of CMBV sweet orange, Nagri (CMBVSON)

Primer Set Nos.	Name of the primer and their sequences	Nucle-tide Nos.	Annea-ling tempera-ture	Exten-sion time	Expected amplicon size based on CMBV sequence accession No. AF 347665 inGen bank
1	CMBV 1F- TGGTATCAGAGCTTGGTTAT CMBV1016- TTGTAAGCGTAGAAGGTA	20 18	54^{0}C	60s	1015 bp
2	CMBV897 F - AACCCCAGCAAGGCTCATCAAC CMBV1972R- CAATCATGTTTCTTGTATCCAC	22 22	54^{0}C	60s	1075bp
3	CMBV 1948F- TGGATACAAGAAACATGATTG CMBV 3440R- GAATCACAAGTAAGCCTCTC	22 20	53^{0}C	90s	1492 bp
4	CMBV3309F- TGATGGTCGTGAGGGTACTCA CMBV 4371R- TCCTGCTGTTGCTGTAAC	21 18	50^{0}C	60s	1062bp
5	CMBV4222F- ACCACTCAGAGAGCTCGCTTACA CMBV 5204 R- CCCAATACTTCATAGGCTCTTC	23 22	53^{0}C	60s	982 bp
6	CMBV 4998 F- CAACACCAGGCTTGCTGCACC CMBV 6205R- CATGCATCCATCCGTTTCG	21 19	50^{0}C	90s	1306 bp
7	CMBV 5894 F - TTCACAAAGGGCTTATCAAG CMBV 6899 R- GCCACCAGTTGTCTTGCTGA	20 20	52^{0}C	60s	1005 bp
8	CMBV 6713 F- AGATTAGATCACCTTTAGCG CMBV 230R- AGATTAGATCACCTTTAGCG	20 20	54^{0}C	60s	~833 bp

PCR amplification

PCR mix and PCR profile used for amplification of CMBV

The composition of PCR mix used for amplification of genome of CMBV is given below,

Sterile distilled water	**31.50 µl**
10 x PCR buffer	**5.00 µl**
25mM MgCl2	**3.00 µl**
10mM dNTPs	**1.00 µl**
Forward primer (100ng/µl)	**2.00 µl**
Reverse primer (100ng/µl)	**2.00 µl**
Taq polymerase (5units/µl)	**0.50 µl**
Template DNA	**5.00 µl**
Total PCR mix volume	**50.00 µl**

For amplification of the target DNA, following PCR profile was used in the Eppendorf Thermal cycler machine AG 22331 (Germany)

94 ^{0}C 5 minutes
94 ^{0}C 30 second
53 ^{0}C - 62^{0}C* 1 minutes } 30 cycles
72 ^{0}C 1 minute
72 ^{0}C 10 minutes

Cloning of amplified products

The vector pDrive 322 (3850bp) (Qiagen, Germany), was used for thecloning of eluted PCR products of CMBVSON. There is a single 3' and 5' U overhang in pDrive322 vector at the insertion sites (multiple cloning sites) which prevent self ligation of the plasmid vector

Ligation and transformation

Qiagen PCR Cloning plus kit (Qiagen, Germany) was used for ligation and Transformation of PCR amplified product

Ligation

i. After thawing 2x ligation master mix and pDrive322 cloning vector DNA, they were placed on ice.

ii. A ligation reaction mixture was prepared according to the following scheme

Component	Volume/reaction
Vector (50ng/µl)	1.0 µl
PCR product	4.0 µl
Ligtaion mix (2X)	5.0 µl
Total	10.0 µl

iii. The ligation reaction mixture was mixed and then incubated for 30 minutes at 4-16 ^{o}C.

iv. The ligation reaction mixture was then used for transformation or stored at -20 ^{o}C until use. (15-18 hours

Transformation

i. Two tubes of Qiagen EZ competent cells were thawed on ice. SOC medium was thawed and brought to room temperature.

ii. 5 µl of ligation reaction mixture was added to the tube of Qiagen EZ competent cells, mixed gently, and incubated on ice for 5 minutes.

iii. The tube was heated in a 42^{o}C in water bath or heating block for 30 seconds without shaking.iv. The tube was then incubated on ice for 2 minutes.

v. 250 µl SOC medium was added to the tube and 100 µl of each transformation mixture was then directly plated on to Luria agar plates containing ampicillin (100 µg /ml), Xgal (80 µg/ml and IPTG (50µm).

V1. The plate was then incubated at room temperature until the transformation mixture had been absorbed in to the agar. The plate and incubate was inverted at 37^{o}C over night

Selection of transformants and screening for the positive clones

The transformants were selected on the basis of blue/white colony colour. The white colonies were selected and subsequently master plates were prepared using Luria agar containing Ampicillin (100 µg/ml), X-gal (200 µg/ml) and IPTG (0.1M) (Sambrook and Russell, 2001). In master plate individual white colonies were streaked in each grid using autoclaved tooth picks. In order to select positive clones carrying the desired insert, colony PCR was done.

Screaming of positive clones by colony PCR

Transferred small amount of bacterial cells from selected recombinant colonies **(**two to four**)** cells from individual grids were using sterile toothpicks to PCR tubes (200 µl) containing the PCR mixture bacterial cells served as template in PCR mix. Amplification conditions described earlier were followed and PCR products were electrophoresed in 1.0% agarose gel containing ethidium bromide.

Nucleotide sequencing of clones and *in silico* analysis

Clones obtained from PCR products of primer sets 2, 3, 4 and5to obtain full length sequence of CMBVSON

Clone	Sequence Number	Expected length (bp)
p-Drive CMBVSON 2	**897F-1972R**	**1075**
p-Drive CMBVSON 3	**1948F-3440R**	**1492 bp**
p-Drive CMBVSON 4	**3309F-4371R**	**1062bp**
p-Drive CMBVSON 5	**4222F-5204 R**	**982bp**

PCR Products obtained with 4 other primers sets 1, 6, 7,8 and used for full genome sequencing of CMBVSON

PCR product	Sequence Number	Length (bp)
CMBV N-1	1F-1016R	~1015 bp
CMBV N-6	4998 F-6205R	~1306 bp
CMBV N-7	5894 F-6899 R	~1005 bp
CMBV N-8	6713 F- 230R	~833 bp

Nucleotide sequencing of clones and *in silico* analysis

Selected recombinant clones of CMBV (CMBVSON-2, CMBVSON-3, CMBVSON-4, CMBVSON-5 with insert of approximate size ~1075, ~1492 bp, ~1062bp, ~982bp (Table 3.2) and PCR Product (CMBVSON-1, CMBVSON-6, CMBVSON-7, CMBVSON-8 (Table 3.3) with ~1015 bp, ~1306 bp, ~1005 bp, ~833 bp sizes were sequenced at Department of Biochemistry, DNA sequencing facility, South Campus, New Delhi

Sequences were analyzed by Blast (http:/www.ncbi.nlm.nih.gov/blast/) (Altschul *et al*., 1997). After sequence comparison checking homology all the sequences were aligned to get full length genome sequence by deleting the one of the overlapping regions between sequences. Protein coding region open reading frame (ORF) was searched by Bioedit as well as by ORF finder available at www.ncbi.nlm.nih.gov/blast/.

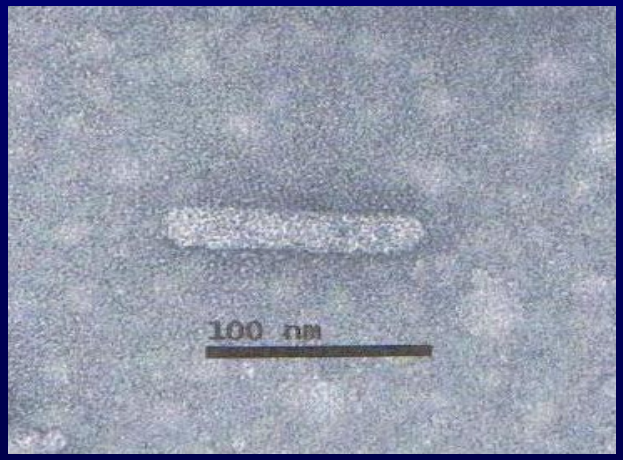
Sequence identity matrix and other basic analysis were carried out using Bioedit Sequence Alignment Editor Version 5.0.9 (Hall, 1999). Multiple sequence alignments were generated using Clustal W (Thompson *et al*., 1994). The full nucleotide and amino acids sequences of other badnaviruses for comparison were obtained from GenBank and multiple alignments at nucleotide and amino acid were generated.

Results

Graft transmission and establishment of culture

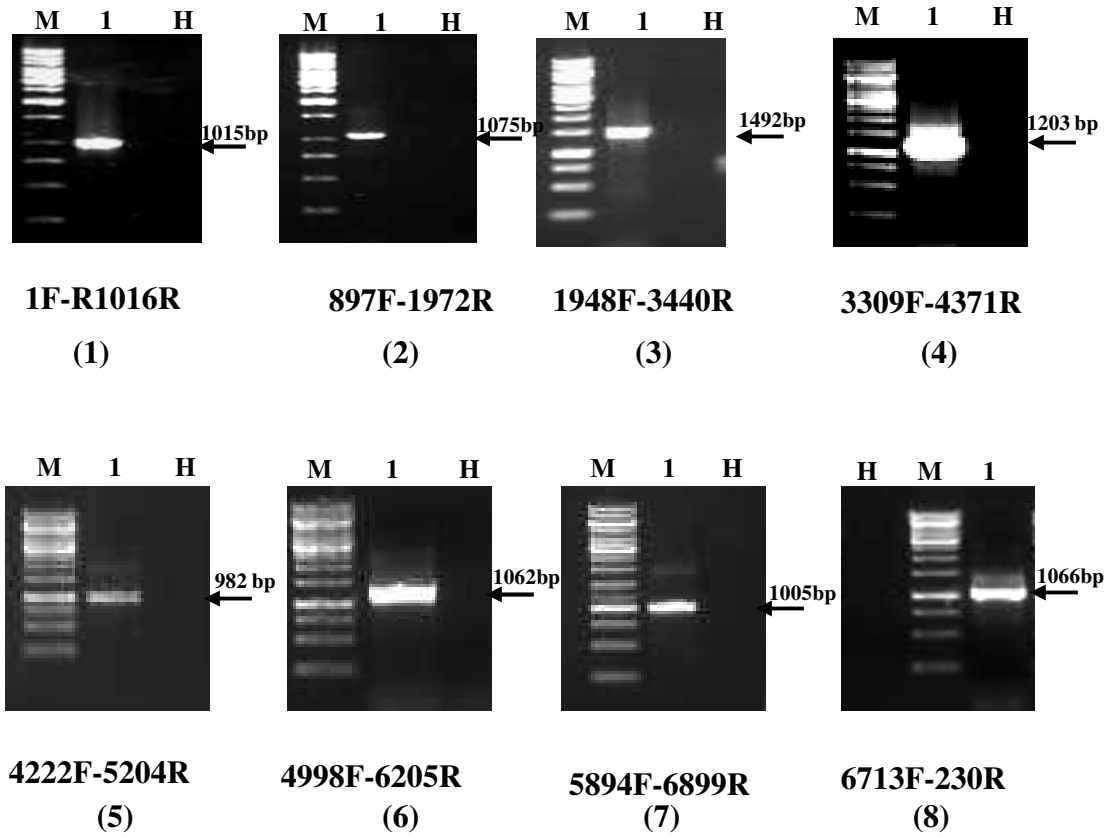
Symptoms developed on sweet orange citrus plant after graft inoculation of *Citrus mosaic virus* infecting sathgudi sweet orange plant .

Electron microscopy of CMBV:



Electron micrograph showing 120x30nm bacilliform particle of *citrus mosaic virus* (CMBV) in leaf dip preparation (magnification 1,00,000

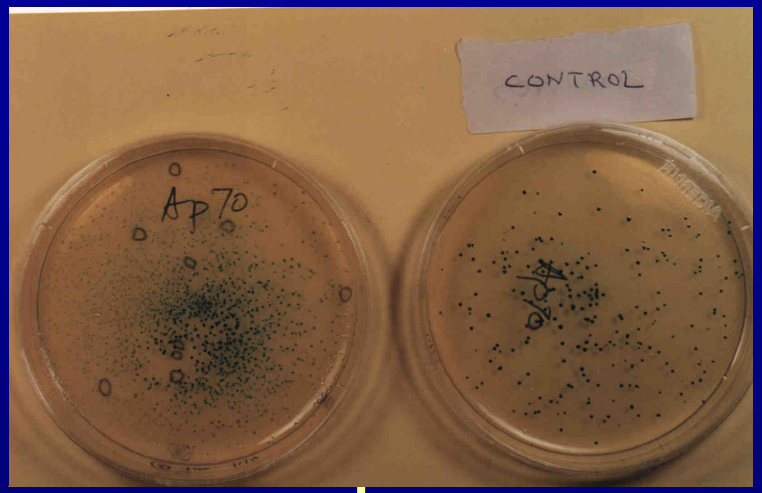
PCR amplification of CMBVSON

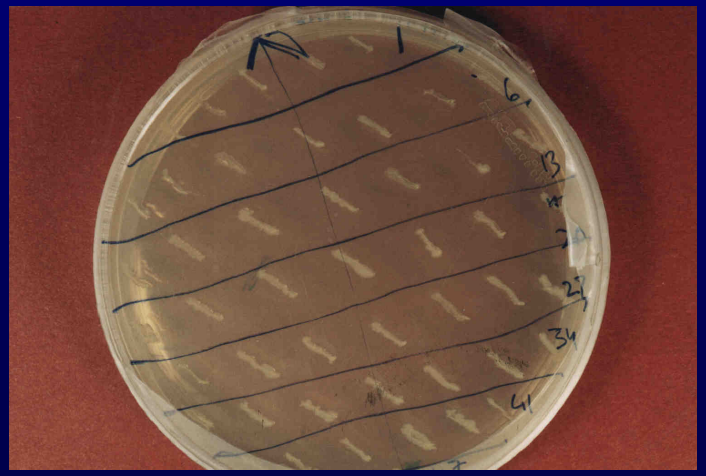


Gel electrophoresis of PCR products of genome of CMBVSON with eight sets of primers

Cloning

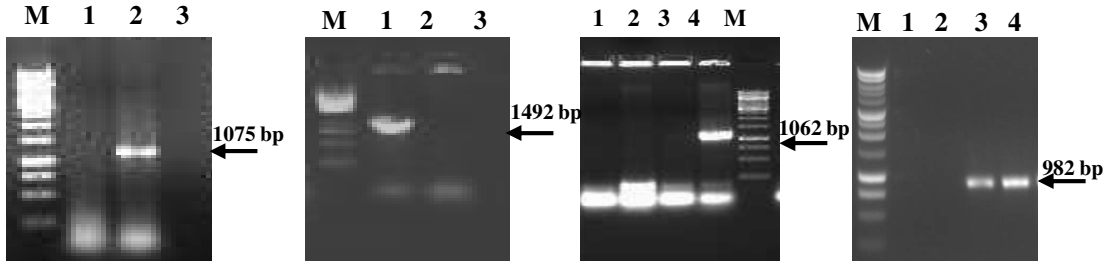
Blue and white colonies





Master plate

Colony PCR



897F-1972R (2)

1948F-3440R (3)

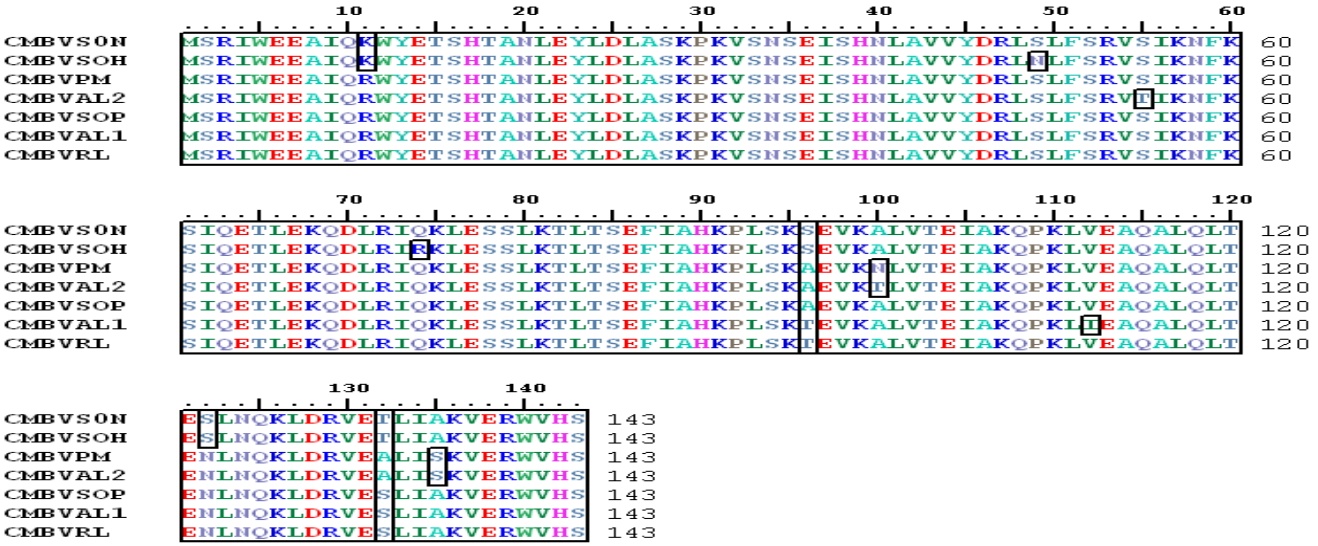
3309F-4371R (4)

4222F-5204R (5)

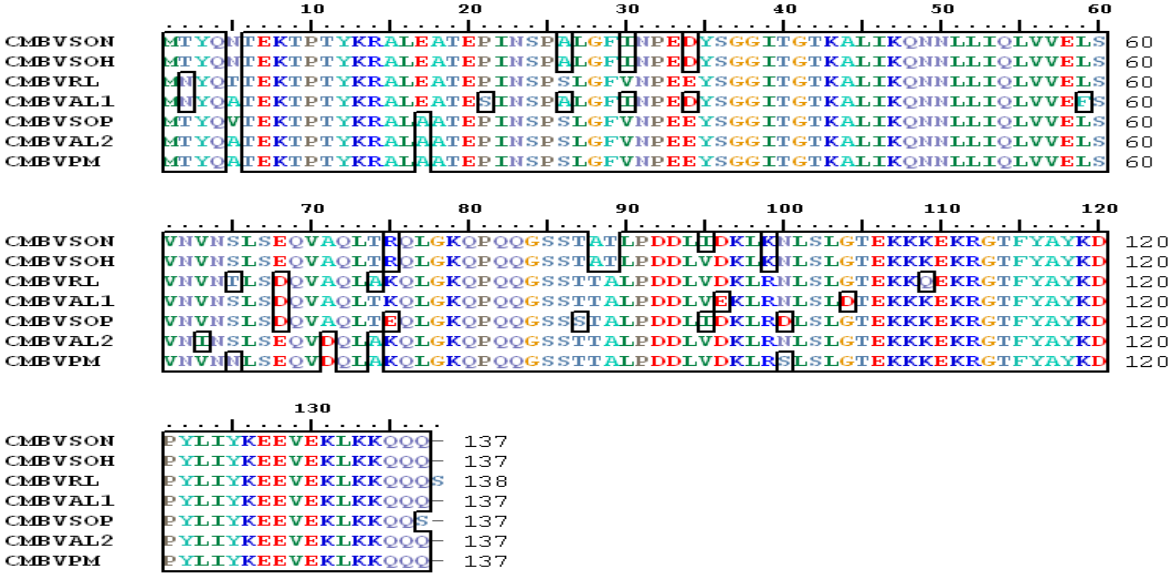
Gel electrophoresis of PCR amplified products from colonies of selected recombinants clones

Multiple alignment of all the six ORFs (Amino acids sequence) of the genome of CMBVSON with other CMBV isolates.

ORF1

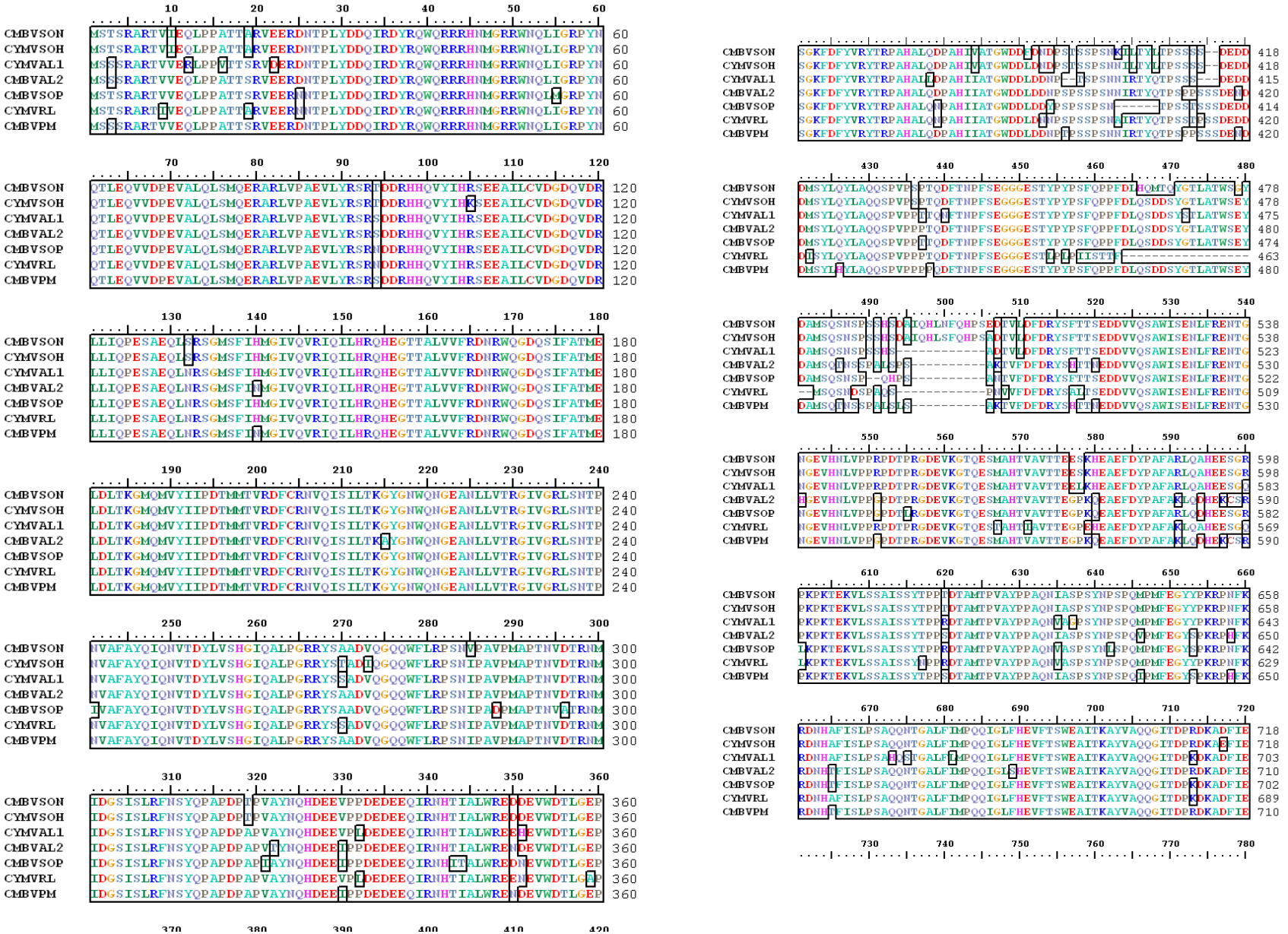


ORF2



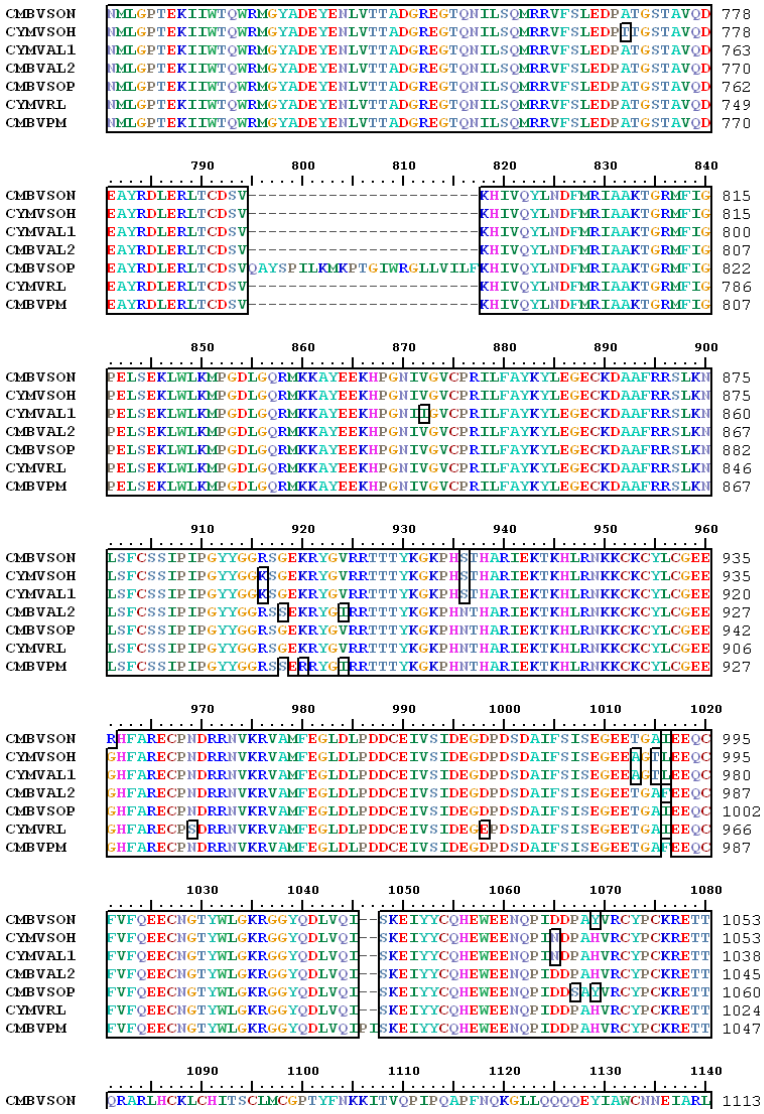
Multiple alignment of all the six ORFs (Amino acids sequence) of the genome of CMBVSON with other CMBV isolates.

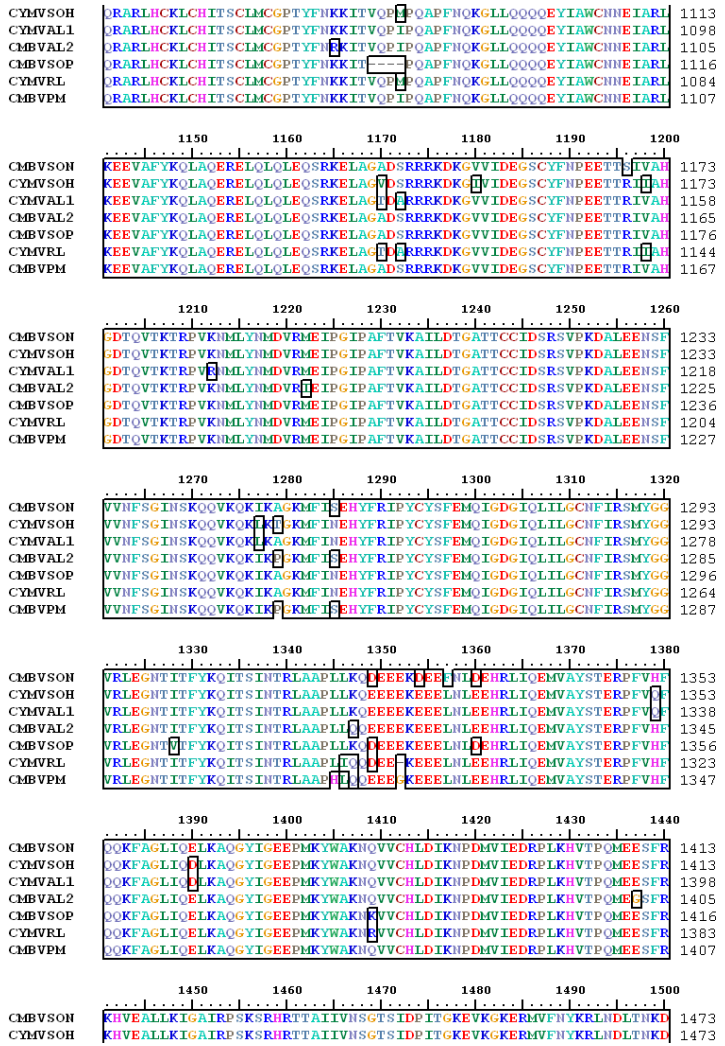
ORF3



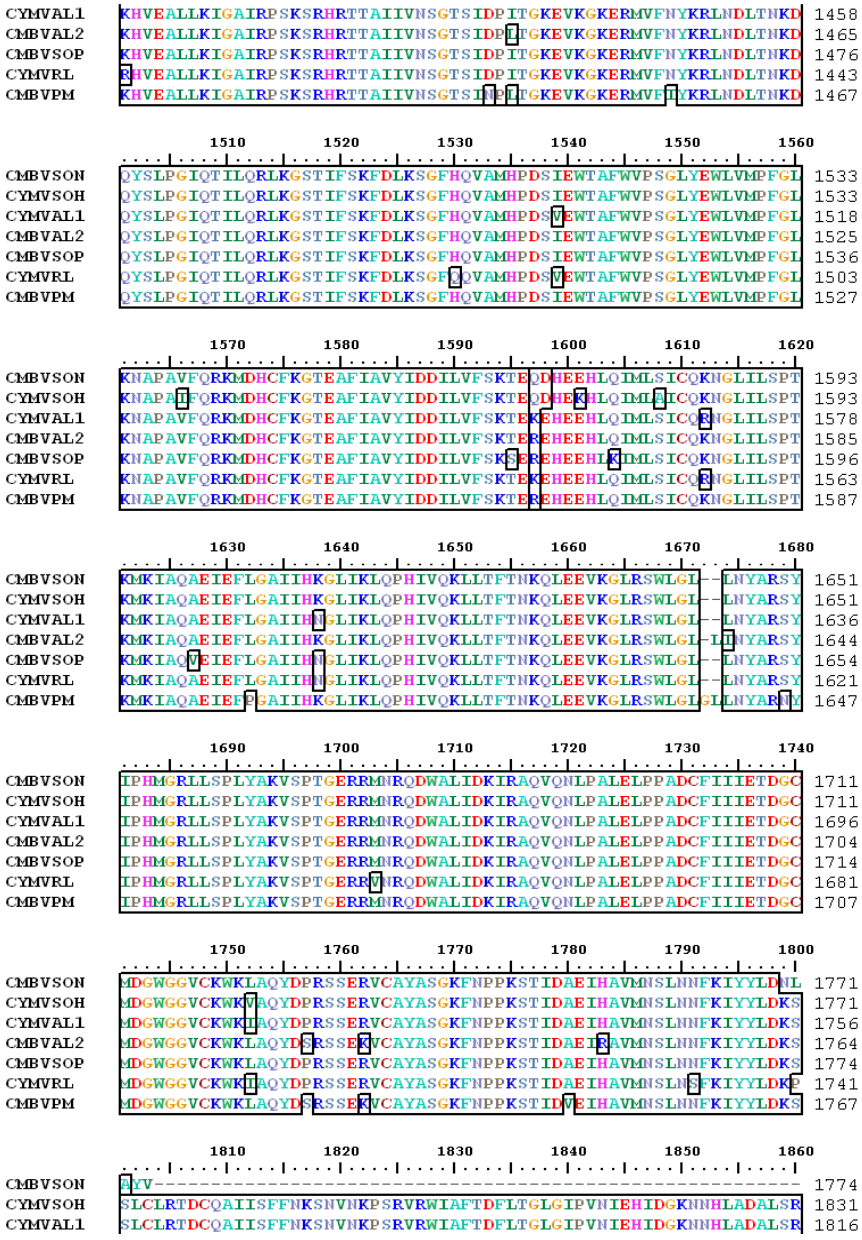
Multiple alignment of all the six ORFs (Amino acids sequence) of the genome of CMBVSON with other CMBV isolates.

ORF3

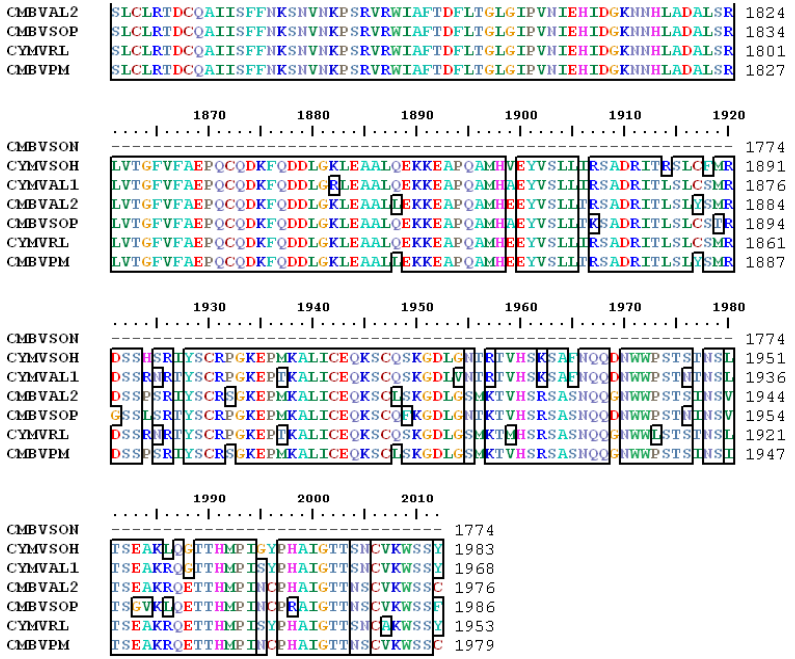




Multiple alignment of all the six ORFs (Amino acids sequence) of the genome of CMBVSON with other CMBV isolates.

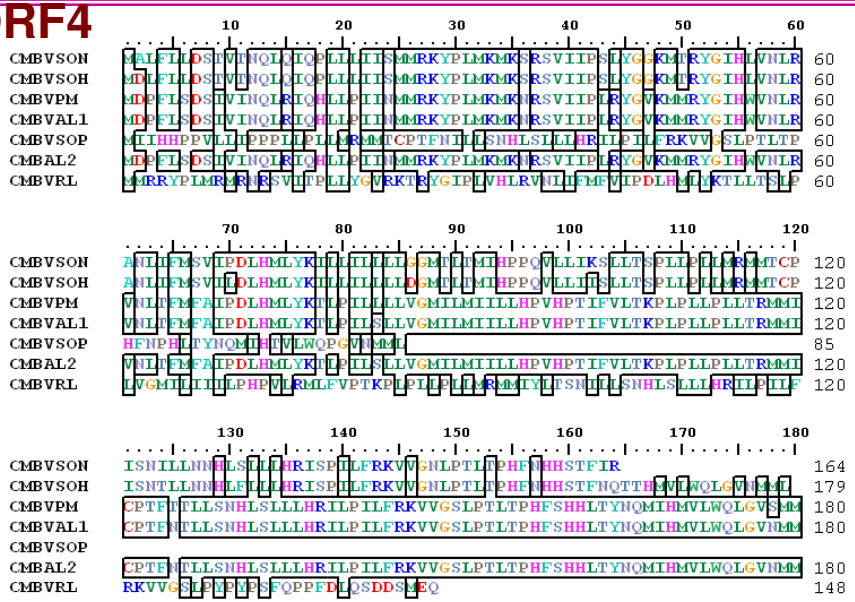


ORF3

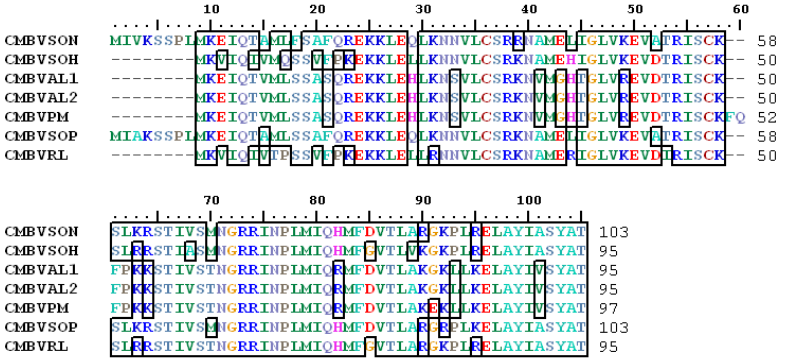


Multiple alignment of all the six ORFs (Amino acids sequence) of the genome of CMBVSON with other CMBV isolates.

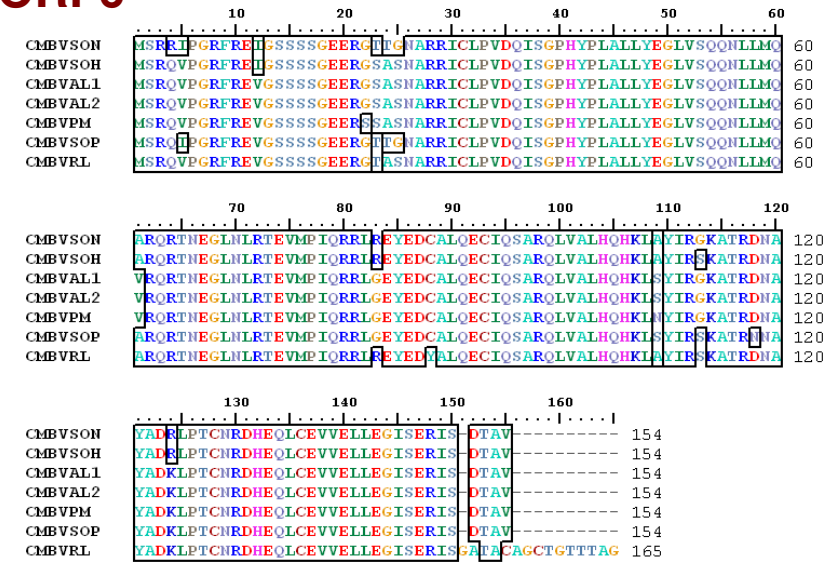
ORF4



ORF5



ORF6



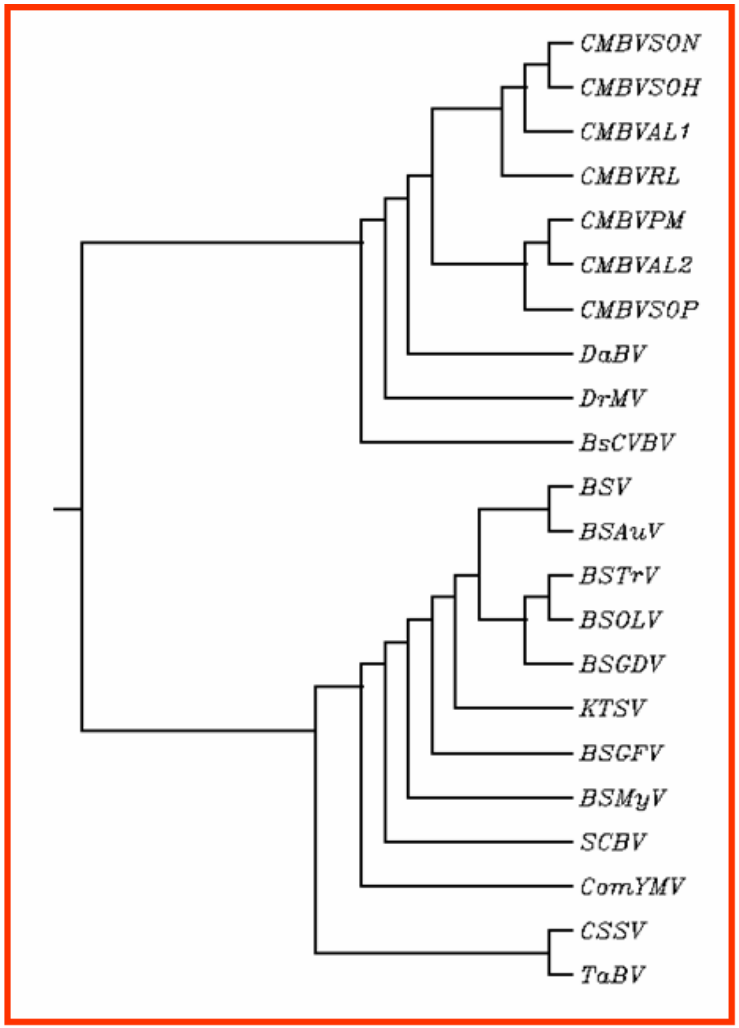
Different ORFs, their location and number of amino acids coded by each ORFs in CMBVSON genome

Name of ORF	Starting nucleotide	Stop nucleotide	Length (bp)	No. of Amino Acids	Molecular weight (kDa)
1	231	662	432	143	14.3
2	659	1072	414	137	13.7
3	1069	6393	5325	1774	17.74
4	1973	2467	495	164	16.4
5	3953	4264	312	103	10.3
6	6593	7057	465	155	15.3

Comparison of full length genome nucleotide sequence of CMBVSON with other badnaviruses

Isolates	CMBV SON	CMBV SOH	CMBVS OP	CMBV AL1	CMBV AL2	CMBV RL	CMBV PM	BSV	BS TRVV	BSAuV	BSGDV	BSGFV	BSMyV	BSOLV	CSVV	KTSV	DrMV	Com YMV	DaBV	SCBV	TaBV
CMBVSON	**100**																				
CMBVSOH	**96.0**	100																			
CMBVSOP	**90.1**	88.1	100																		
CMBVAL1	**91.7**	93.0	88.1	100																	
CMBVAL2	**87.7**	87.6	90.2	88.0	100																
CMBVRL	**89.1**	88.9	89.9	91.6	88.2	100															
CMBVPM	**87.5**	87.4	90.2	87.8	98.9	88.6	100														
BSV	**36.7**	36.5	36.2	36.9	36.5	36.9	36.9	100													
BSTrV	**36.2**	36.4	36.3	36.4	36.1	36.3	36.2	53.2	100												
BSAuV	**36.7**	36.9	36.7	36.9	36.5	36.9	36.5	100	53.3	100											
BSGDV	**36.2**	36.4	36.3	36.4	36.2	36.3	36.2	53.2	99.7	53.2	100										
BSGFV	**36.8**	37.0	36.6	37.0	36.4	36.9	36.5	48.6	47.5	48.6	47.4	100									
BSMyV	**38.3**	38.4	38.3	38.2	38.0	38.6	38.2	50.0	48.4	50.0	48.4	48.8	100								
BSOLV	**37.7**	37.9	37.7	37.9	37.6	37.8	37.7	55.0	93.1	55.0	93.0	43.0	49.7	100							
CSSV	**48.4**	48.6	48.9	49.1	48.9	48.8	49.0	37.4	36.8	37.4	36.8	38.0	38.1	38.3	100						
KTSV	**36.9**	37.0	36.7	36.8	36.4	36.7	36.4	53.4	53.2	53.4	53.2	47.8	47.8	55.3	37.3	10.0					
DrMV	**42.2**	42.1	41.7	42.3	41.4	42.5	41.7	38.7	37.0	38.7	36.9	39.0	39.3	38.6	38.9	38.3	100				
ComYMV	**37.3**	37.3	37.5	37.2	37.7	37.2	37.7	41.6	41.6	41.6	41.5	40.7	41.9	42.2	38.6	40.7	36.3	100			
DaBV	**48.2**	48.4	47.9	48.2	48.3	48.2	48.2	36.3	35.6	36.3	35.3	36.7	37.2	36.2	45.4	36.6	39.3	39.6	100		
SCBV	**35.2**	34.9	35.3	35.2	35.1	35.2	35.0	45.2	43.9	45.2	43.9	51.0	45.8	45.2	36.3	47.3	37.0	40.3	34.7	100	
TaBV	**38.0**	38.1	38.1	38.1	37.9	38.1	37.9	37.3	36.6	37.3	36.6	38.3	38.3	38.2	39.1	38.0	41.0	34.9	36.1	36.7	100

- **96% similarity with CMBVSOH**
- **48.4 % similarity with CSSV**



Phylogenetic tree of full length genomes CMBVSON other badnaviruses

Percent identity of nucleotide sequence and **ORFs** encoded by them in different of CMBV isolates and their comparison with other badnaviruses

Viruses / CMBV Isolates	Nucleotide Sequence Identity %	ORFI		ORFII		ORFIII		ORFIV		ORFV		ORFVI	
		Size (aa)	Identity %	Size (aa)	Identity %	Size (aa)	Identity %	Size (aa)	Identity %	Size (aa)	Identity %	Size (aa)	Identity %
CMBVSOH	87.4 – 96.0	143	95.1 – 98.6	137	89.7 – 99.2	1983	87.1 – 95.9	178	34.6 – 87.1	95	71.1 – 90.5	154	89.6 – 96.1
CMBVSON	87.5 – 96.0	143	95.1 – 98.6	137	89.1 – 99.2	1744	83.7 – 87.1	164	28.4 – 87.1	103	69.5 – 95.1	153	87.2 – 96.1
CMBVSOP	88.1 – 90.2	143	97.2 – 99.3	137	89.0 – 92.7	1967	83.7 – 93.8	85	28.4 – 38.6	103	71.4 – 95.1	154	87.8 – 95.4
CMBVAL1	87.8 – 93.0	143	95.1 – 99.3	137	89.0 – 91.9	1968	84.9 – 95.9	181	38.6 – 100	95	71.5 – 96.9	154	88.4 – 100
CMBVAL2	87.6 – 98.9	143	95.1 – 98.6	137	90.5 – 97.8	1976	84.4 – 98.6	181	38.6 – 100	95	71.5 – 96.9	154	88.4 – 100
CMBVRL	88.6 – 91.6	143	95.8 – 99.3	138	89.1 – 93.4	1954	83.4 – 94.3	148	29.9 – 55.8	95	69.0 – 90.5	154	87.2 – 89.6
CMBVPM	87.4 – 98.9	143	94.4 – 98.6	137	89.7 – 97.8	1979	84.2 – 93.8	181	37.5 – 98.3	97	69.5 – 96.9	154	87.8 – 98.7
Other badnaviruses													
CSSV	48.4 – 49.1	143	53.8 – 55.9	145	18.2 – 19.4	1816	49.3 – 50.3	113	03.0– 07.2	131	6.8–9.1	-	-
BSV	36.5 – 36.9	176	19.8 – 20.4	132	19.7 – 21.7	1900	30.7 – 33.0	-	-	-	-	-	-
BSAuV	36.5 – 36.9	176	19.8 – 20.4	132	19.7 – 21.7	1900	30.7 – 33.0	-	-	-	-	-	-
BSTrV	36.1 – 36.4	176	22.0 – 22.5	134	19.4 – 20.8	1709	33.8 – 34.5	-	-	-	-	-	-
BSGDV	36.2 – 36.4	177	22.0 – 22.5	134	19.4 – 20.8	1709	33.8 – 34.5	-	-	-	-	-	-
BSGFV	36.4 – 37.0	177	19.3 – 19.8	134	17.4 - 18.1	1832	32.6 – 34.7	-	-	-	-	-	-
BSMyV	38.2 – 38.6	176	19.8 – 20.4	132	19.7 – 21.7	1900	30.7 – 33.0	-	-	-	-	-	-
BSOLV	37.6 – 37.9	175	22.0 – 22.5	112	19.3 – 20.8	1832	31.8 – 34.0	-	-	-	-	-	-
BsCVBV	33.6 – 34.2	132	16.3 – 16.9	147	16.0 – 17.3	2252	28.1 – 30.4	198	04.0 – 07.0				
ComYMV	37.2 – 37.7	200	17.3 – 17.8	135	12.9 – 14.9	1886	33.4 – 33.8	-	-	-	-	-	-
DaBV	47.9 – 48.4	143	34.4 – 35.8	125	31.8 – 34.7	1295	24.4 – 27.8	59	03.0 – 0.03	-	-	-	-
DrMV	41.6 – 42.6	149	34.6 – 35.3	131	25.3 – 26.9	1916	33.6 – 36.5	103	09.0 – 12..1	91	04–13.8	139	21.2–22.7
KTSV	36.4 – 37.0	173	14.7 – 15.3	124	20.5 –22.6	1941	30.0 – 32.5	-	-	-	-	-	-
SCBV	34.9 – 35.3	185	16.4 – 17.0	123	21.1 – 24.6	1912	26.8 – 28.9	-	-	-	-	-	-
TaBV	37.9 – 38.1	146	33.0 - 34.6	144	21.9 – 23.9	1881	30.7 – 33.7	-	-	-	-	-	-

Conclusions

1. A badnavirus designated as CMBVSON was confirmed in sathgudi sweet orange showing mosaic symptoms by electron microscopy and PCR.
2. The complete genome of CMBVSON consisted of 7558 nucleotide containing for 6 open reading frames (ORFs). High variability (87.4% to 96%) observed in ORF 4 and 5 infecting sathgudi sweet orange, rangpur lime acid lime and pummelo.
3. The intergenic region (non coding region) has 731 nucleotide. Intergenic region consists of putative promoter elements like TATATAA box, CACAAT sequence, TGACG sequence and AATAAA the polyadenylation signal sequence.
4. The molecular weight of full genome of CMBV sathgudi sweet orange isolate is 247.60 kDa with G+C Content 43.52% and A+T content 56.48%. The (A+T) and (G+C)ratio was 1.29.
5. The genome consists, Movement protein ,Aspartic Protease Reverse transcriptase and RNase H in ORF3.
6. Sequence information will also be useful in designing primers for detection of different isolates of CMBV in PCR and production disease free planting materials of citrus.

Thank You

Base composition of CMBVSON and its comparison with other CMBV isolates of citrus

CMBV Isolates	Base Composition				A+T (%)	G+C (%)	Molecular Weight in KDa	Total Nucleotides (bp)
	A	T	G	C				
CMBVSON	2326	1943	1668	1621	56.48	4352	4586.7	7558
CMBVSOH	2337	1925	1666	1631	56.38	43.62	4586.9	7559
CMBVSOP	2322	1922	1666	1587	56.61	43.39	4549.0	7497
CMBVAL1	2312	1894	1675	1617	55.21	45.79	4549.0	7498
CMBVRL	2321	1620	1671	1910	56.25	43.75	4562.1	7522
CMBVAL2	2323	1877	1653	1620	56.19	43.79	4539.0	7473
CMBVPM	2323	1889	1648	1616	56.34	43.66	4541.0	7487

Comparison of ORFs of CMBVSON with CMBVSOH