ResearchGate

See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/285830071

Biological and molecular characterization of Bean common mosaic virus associated with vanilla in India

Article *in* Indian Journal of Virology · July 2009

CITATIONS
4

READS
48

2 authors:

Bhadra Murthy Vemulapati
Koneru Lakshmaiah University
9 PUBLICATIONS **62** CITATIONS

SEE PROFILE

Alangar Ishwara Bhat
Indian Institute of Spices Research
79 PUBLICATIONS **500** CITATIONS

SEE PROFILE

All in-text references underlined in blue are linked to publications on ResearchGate, letting you access and read them immediately.

Available from: Bhadra Murthy Vemulapati
Retrieved on: 17 September 2016

Indian Journal of Virology (2009), 20(2): 70-77
Available on line at www.indianjournals.com / www.iospress.nl

Research Article | Plant Virus

Biological and molecular characterization of *Bean common mosaic virus* associated with vanilla in India

V. BHADRAMURTHY and A. I. BHAT
Division of Crop Protection, Indian Institute of Spices Research, Calicut 673 012

Received 13 March 2009; Accepted 01 September 2009

ABSTRACT

Bean common mosaic virus (BCMV) associated with vanilla (*Vanilla planifolia* Andrews) in India was characterised based on host reaction and coat protein (CP) sequence properties. The virus caused mosaic, leaf and stem necrosis, leaf distortion and stunting followed by drying of aerial adventitious roots. Leaf-dip preparation of infected vanilla leaves revealed flexuous filamentous particles characteristic of a *Potyvirus*. In mechanical inoculation tests, the virus was transmitted to plant species under family Chenopodiaceae, Fabaceae and Solanaceae. *Vigna unguiculata* and *Nicotiana benthamiana* were found suitable propagation hosts of BCMV. The virus was partially purified from inoculated *V. unguiculata* leaves and the molecular weight of the viral coat protein was measured to be 34 kDa. Partial NIB and CP gene (c.a. 850 bp) of the virus was amplified using reverse transcriptase-polymerase chain reaction (RT-PCR) and finally cloned and sequenced. The cp gene contained 823 nucleotides potentially coding for 274 amino acids. The present BCMV isolate showed 87-96% nucleotide and 87-98% amino acid sequence identities with other BCMV isolates/strains. The isolate showed maximum identity of 96% and 98% at nucleotide and amino acid level, respectively, with Blackeye (Blk1) strain of BCMV indicating its closeness to cowpea infecting BCMV isolates. This is the first report of occurrence of BCMV on *V. planifolia* from India.

Bhadramurthy V. and Bhat A. I. 2009. Biological and molecular characterization of *Bean common mosaic virus* associated with vanilla in India. *Indian J. Virol.* 20(2): 70-77.

Corresponding author: A. I. Bhat (aib65@yahoo.co.in)
Keywords: *Bean common mosaic virus*, coat protein gene, host range, identification, RT-PCR, sequence analyses, serological detection, *Vanilla planifolia*

INTRODUCTION

Bean common mosaic virus (BCMV) (genus: *Potyvirus*, family: Potyviridae) is economically the most important virus infecting bean crops world wide. It is aphid and seed-borne transmitted virus and represents a complex of virus strains (Khan *et al*., 1993; Mink *et al*., 1994). BCMV is a monopartite flexuous rod shaped virus with positive sense ssRNA genome of about 10 kb size. Earlier, identification and strain differentiation of BCMV were done based on their response on a series of *Phaseolus vulgaris* host differential cultivars (Drifhout, 1978). Later, on the basis of serological reactivity, isolates/strains of BCMV were divided into serogroups A and B (Vetten *et al*., 1992). Recent phylogenetic analysis established that viruses from these two different serotypes actually belong to two different species, where members of serotype A were placed in the species, *Bean common mosaic necrosis virus* (BCMNV) and members of serotype B were placed in the species, BCMV (Berger *et al*., 1997).

Vanilla (*Vanilla planifolia* Andrews) is a valuable orchid spice cultivated for its highly priced beans. In India, large scale intensive cultivation of vanilla started only recently. Viral diseases have become serious problem due to the recent intensive cultivation and so far occurrence of two viruses namely, *Cucumber mosaic virus* (Madhubala *et al*., 2005) and *Cymbidium mosaic virus* (Bhat *et al*., 2006) were reported from India. Globally vanilla is infected by ten different viruses (Grisoni *et al*., 2006). Although occurrence of *Potyvirus* like particles based on leaf dip electron microscopy was reported from India (Bhat *et al*., 2004), an authentic identification of the causal virus remained unaddressed. In this article, we report the occurrence of BCMV on *V. planifolia* in India on the basis of biological and coat protein (CP) gene sequence properties.

MATERIALS AND METHODS

Virus isolates

A total of 30 vanilla plants exhibiting virus-like symptoms such as mosaic, leaf and stem necrosis, leaf distortion, stunting and

drying of aerial adventitious roots were collected from Karnataka and Kerala and these symptomatic plants were served as initial source of virus inoculum. These samples were initially subjected to direct antigen coated enzyme linked immunosorbent assay (DAC-ELISA) using *Potyvirus* group specific antiserum (Agdia, USA).

Mechanical inoculation

A vanilla plant collected from Calicut, Kerala that reacted positively to the polyclonal antiserum was mechanically inoculated onto *Chenopodium amaranticolor*. Mechanical inoculation was carried out in pre-chilled pestle and mortar by extracting sap in cold 0.1 M phosphate buffer (pH 7.2) containing 0.1% (v/v) 2-mercaptoethanol. The extracted sap was inoculated on the leaves of healthy test plants dusted with celite and then washed off with tap water after 2-3 min.

Host range

Pure culture of the virus maintained on *C. amaranticolor* was used as source of virus inoculum. A total of 19 different plant species belonging to five families grown in pots under insect-proof condition were mechanically inoculated and observed for symptom expression (Table 1). The symptoms were recorded and viral etiology was confirmed by back inoculation test onto *C. amaranticolor*.

Virion Properties

Electron microscopy of leaf-dip preparation was carried out using 2% uranyl acetate (pH 4.5) as negative stain and examined under JEOL-100-CF-II transmission electron microscope at the Unit of Virology, Division of Plant Pathology, Indian Agricultural Research Institute, New Delhi. Virus was propagated on *V. unguiculata* cv. C-152 plants by mechanical inoculation and leaves harvested two weeks after inoculation was used for purification. Partial purification of the virus was carried out by the protocol of Gough and Shukla (1981) with a modification that included addition of urea (0.2 M) in the extraction buffer. Polyclonal antiserum against the virus was produced in a New Zealand White rabbit by injecting partially purified virus preparation (0.5 ml of virus with Freund's incomplete adjuvant, 1 : 1) intramuscularly four times at regular weekly intervals. The animal was bled 14 days after the last injection and the antiserum was collected.

To determine coat protein molecular weight, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out using 12% resolving gel and 5% stacking gel as described by Laemmli (1970). The partially purified virus

Table 1: Reaction of different plant species to *Bean common mosaic virus* infecting vanilla

Family	Plant species	Symptoms*	Days taken for symptom expression	Plants with symptoms/ Total plants inoculated
Chenopodiaceae	*Chenopodium amaranticolor*	NLL	3-7	20/20
Cucurbitaceae	*Benincasa hispida*	AS	-	0/10
	Cucumis sativus	AS	-	0/10
	Cucurbita pepo	AS	-	0/10
	Momordica charantia	AS	-	0/10
Fabaceae	*Cicer arietinum*	AS	-	0/10
	Glycine max	AS	-	0/10
	Canavalia ensiformis	AS	-	0/10
	Phaseolus vulgaris	AS	-	0/20
	Phaseolus lunatus	AS	-	0/20
	Canavalia gladiata	AS	-	0/20
	Cajanus Cajan	AS	-	0/10
	Vicia faba	AS	-	0/20
	Vigna unguiculata cv. C-52	Bl, M, LD, VB	7 - 10	20/20
	cv. C152	Bl, M, LD, VB	7 - 10	20/20
	cv. Kanakamani	Bl, M, LD, VB	7 - 10	20/20
	cv. Lola	MM	7 - 10	20/20
	cv. Co-6	AS	-	0/20
	cv. Co-7	AS	-	0/20
Solanaceae	*Capsicum annuum*	AS	-	0/20
	Nicotiana benthamiana	Bl, M, Mo, LD	7-10	20/20
	N. glutinosa	AS	-	0/10
	N. tabacum var. Samsun	AS	-	0/10
	N. tabacum var. Xanthii	AS	-	0/10
Orchidaceae	*Vanilla planifolia*	M, CS, N, TL	4 - 6 months	2/5

*AS-asymptomatic, Bl-blistering, CS-chlorotic streaks, LD-leaf deformation/distortion, M-mosaic, MM-mild mosaic, Mo-mottling, N-necrosis, NLL-necrotic local lesion, TL-thinning of leaf, VB-vein banding.

preparation was mixed with an equal volume of sample buffer and denatured for 3 min in a boiling water bath and then the sample was electrophoresed on the gel. The marker protein (Fermentas, USA) was used as size standard.

Electroblot immuno assay (EBIA) after SDS-PAGE was performed in nylon membrane as described in O'Donell *et al.* (1982). Homologous antiserum against the virus isolate was cross-absorbed using healthy cowpea sap. Required quantity of antiserum (1:250 dilution) was incubated with healthy cowpea sap diluted in PBS (1:50) for 1 h at 37 °C. The mixture was centrifuged at 8000 rpm for 5 min. The supernatant was used as primary antibody for probing the membrane. Anti-rabbit IgG labelled with alkaline phosphatase (Genei, Bangalore) was used at 1:10000. Pre-stained marker protein (Fermentas, USA) was used as size standard.

Primer design and synthesis

Seven primer pairs specific to seven different potyviruses known to infect vanilla world wide were designed (Table 2) based on multiple sequence alignment of nuclear inclusion b (NIb) and coat protein (CP) gene sequences of respective viruses available from GenBank.

Total RNA extraction

Total RNA was isolated following the method described by Siju *et al.* (2007) with a few modifications. In this method 75 mg of tender infected vanilla leaf tissue was ground in 500 µl of extraction buffer (4 M guanidium thiocyanla, 25 mM sodium citrate and 0.5% sodium *N*-lauryl sarcosine) containing 1% (v/v) 2-mercaptoethanol. To this homogenate, 50 µl of 2 M sodium acetate (pH 4.0), 500 µl of water-saturated phenol and 100 µl of chloroform: isoamyl alcohol (49:1) was serially added with intermittent mixing between each step. After the addition of chloroform: isoamyl alcohol (49:1), the microfuge tube was vigorously mixed for 15 s and incubated on ice for 15 min. The resulting homogenate was centrifuged at 12000 *g* for 15 min at 4°C. Equal volume of isopropanol was added to the aqueous phase collected in a new microfuge tube. The mixture was incubated at -20°C for 1 h and centrifuged at 12000 *g* for 15 min at 4°C. The supernatant was discarded and pellet was washed with 300 µl of 70% ethanol and centrifuged at 12000 *g* for 5 min at 4°C. The supernatant was removed and pellet was air-dried and dissolved in 50 µl of ultra pure water.

Reverse transcriptase polymerase chain reaction (RT-PCR)

RT and PCR were performed as described by Pappu *et al.* (1993) in the same tube without any buffer changes in between. The PCR mixture contained 50 ng each of the sense and antisense primers (details given in Table 2), 10 U Ribonuclease inhibitor (Fermentas, USA), 20 U M-MuLV reverse transcriptase (Fermentas, USA), 1.5 U *Taq* polymerase (New England Biolabs, U.S.A.), 1 × PCR buffer (Genei, Bangalore), 10 mM dithiothreitol (Genei, Bangalore) and 10 µM each of the dNTPs (Sigma, USA). PCR mixture (46 µl) containing the above components was added to the tubes containing the template RNA (4 µl) resulting in a final reaction volume of 50 µl. Amplification was performed in an automated thermal cycler (Eppendorf Master Cycler Gradient) and the program consisted of one cycle at 42°C for 45 min for cDNA synthesis followed by a 40 cycle reaction profile involving 30 s of denaturation at 94°C, 1 min of annealing at 50°C and 1 min of extension at 72°C and a single cycle of final extension at 72°C for 10 min. The reaction products (25 µl) were analysed on 1% agarose gel along with 500 bp

Table 2: Details of primers synthesized and used in the amplification of potyviruses infecting vanilla. [Universal degenerate codes for bases: R(A/G) Y(C/T) M(A/C) K(G/T) S(G/C) W(A/T) H(A/T/C) B(G/T/C) V(G/A/C) D(G/A/T) N(A,C,G,T)]

Primer	Sequence (5' to 3')	Orientation	Primer Type	Virus* and region used	Expected product (bp)
AIB88	GCACCATATAGTCAATTGAG	Sense	Specific	BYMV - 3' end of NIb to 3' end of CP gene	950
AIB89	GACATCTCCTGCTGTGTG	Antisense	Specific		
AIB90	GGATGCGGAGAATCTGTG	Sense	Specific	BCMV - 3' end of NIb to 3' end of CP gene	850
AIB91	GATTGACGTCCCTTGCAG	Antisense	Specific		
AIB92	GGAATTAGCTAGTCTTGGAA	Sense	Specific	CABMV - 3' end of NIb to 3' of CP gene.	950
AIB93	GTCATCCCTAAGAGGGAG	Antisense	Specific		
AIB94	CTATCTRTGGCTRCTTGAAA	Sense	Degenerate	DsMV - 3' end of NIb to 3' of CP gene.	900
AIB95	GGTGTSACGTCCTTTGCA	Antisense	Degenerate		
AIB96	GGCTTCTAAGCAAAGATGAA	Sense	Specific	WMV - 3' end of NIb to 3' of CP gene.	950
AIB97	GTCCCTTGCAGTGTGCC	Antisense	Specific		
AIB109	GGCGCATTTCTCAAACGC	Sense	Specific	OrMV - CP gene.	330
AIB110	CACTAAATCACATATTAACAC	Antisense	Specific		
AIB111	GGATGCAGGAAGAGAATC	Sense	Specific	WVMV - CP gene.	425
AIB112	CATAACCCAAACACCATTC	Antisense	Specific		

Annealing temperature employed varied from 50-56°C.

*BYMV: *Bean yellow mosaic virus;* BCMV: *Bean common mosaic virus;* CABMV: *Cowpea aphid-borne mosaic virus;* DsMV: *Dasheen mosaic virus;* WMV: *Watermelon mosaic virus;* OrMV: *Ornithogalum mosaic virus;* WVMV: *Wisteria vein mosaic virus.*

DNA ladder (Fermentas, USA). The DNA bands were visualized and photographed using a UV transilluminator and a gel documentation apparatus (Alpha Innotech Corporation, CA, USA).

Cloning, sequencing and sequence analyses

The PCR product obtained was purified using Perfectprep Gel Cleanup Kit (Eppendorf, Germany) following manufacturers' instructions and cloned into pTZ57R/T cloning vector (Fermentas, USA) and transformed into competent *Escherichia coli* strain JM-109 using InsTAclone PCR Cloning Kit (Fermentas, USA) following manufacturers' instructions. Recombinant clones were identified by PCR as well as restriction endonuclease digestion and selected clones were sequenced at the automated DNA sequencing facility available at Genei, Bangalore. Sequence data were compiled using Seqaid Version 3.6 (Rhoads and Roufa, 1985). Multiple sequence alignments were made using Clustal X (1.81). Per cent sequence identities were determined using Bioedit program version 5.0.9. Sequence phylogram was constructed by Maximum Parsimony Bootstrap method (Bootstrap analysis with 500 replicates) in MEGA (4.1). The CP nucleotide and amino acid sequences of other BCMV isolates used for comparison (Table 3) were obtained from GenBank (Benson *et al*., 1999). The BLAST programme (Altschul *et al*., 1997) was used to identify related sequences available from the GenBank database.

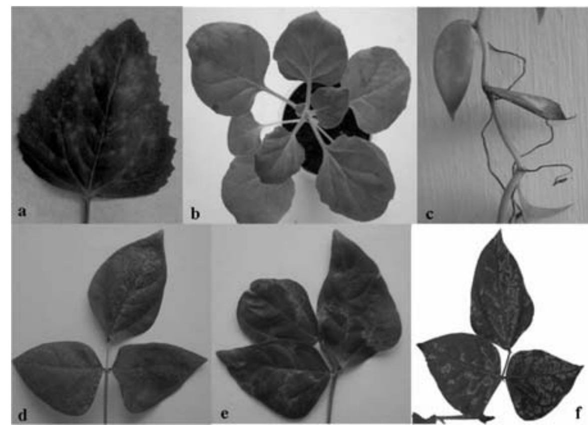

Fig. 2: *Test plants exhibiting different kinds of symptoms upon mechanical inoculation with virus isolate under green house conditions. (a) Chenopodium amaranticolor with local lesions (b) Nicotiana benthamiana with mosaic (c) Vanilla planifolia with mosaic and necrosis (d-f) Vigna unguiculata cvs. C-52, C-152 and Kanakamani with mosaic, blisters and leaf deformation.*

RESULTS

Symptomatology and host range

The characteristic disease symptoms associated with BCMV infection in vanilla included mosaic, leaf and stem necrosis, leaf distortion and stunting. The plants also exhibited drying of aerial adventitious roots (Fig. 1). Mechanical inoculation of the sap obtained from such symptomatic tissues produced local lesions on *C. amaranticolor* (Fig. 2a). Pure culture of the virus was established by inoculation of the sap obtained from single local lesion. This was used as source of virus inoculum for all the subsequent studies. Similar kind of symptoms (as observed under field conditions) were produced on *V. planifolia* upon back inoculation from *C. amaranticolor* (Fig. 2c) thus confirming pathogenicity of the virus. In host range studies the causal virus

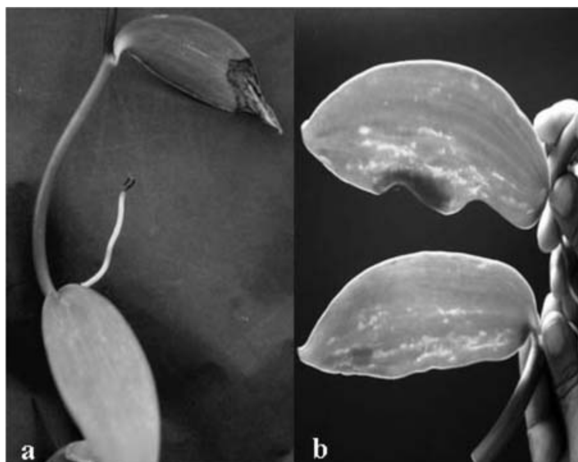

Fig. 1: *(a) BCMV infected vanilla plant exhibiting necrosis of leaf and stem. (b) mosaic and deformation of vanilla leaf.*

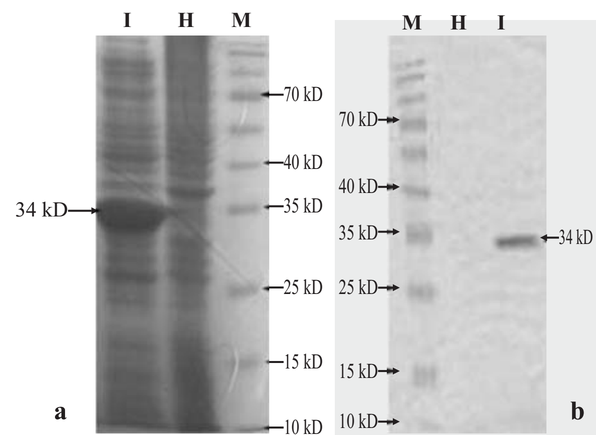

Fig. 3: *(**a**) SDS-PAGE analysis of coat protein of partially purified virus. Lane I: Partially purified virus preparation; Lane H: healthy cowpea; Lane M: marker protein and numbers on right indicate MW of marker proteins. (**b**) EBIA analysis of partially purified preparations. Lane M: Pre-stained marker proteins (Fermentas) and numbers on left indicate MW of marker proteins; Lane H: healthy cowpea; Lane I: partially purified virus preparation. Blotted protein was probed with cross adsorbed homologous antiserum of BCMV.*

infected *N. benthamiana* and *V. unguiculata* (cvs. C-52, C-152, Kanakamani) (Fig. 2d-e). The symptoms produced by each host species is provided in Table 1. None of the plant species tested from *Cucurbitaceae* was found susceptible.

Virion properties

Leaf dip electron microscopy revealed the presence of typical flexuous filamentous particles, characteristic of potyviruses. In purification, sucrose density gradient centrifugation did not show any visible light scattering zone. However the pellet obtained after ultracentrifugation (used to load onto sucrose gradient) when checked under electron microscope showed flexuous particles and its infectivity confirmed by mechanical inoculation onto *C. amaranticolor*. The partially purified virus upon electrophoresis on SDS-PAGE, a major band corresponding to 34 kDa was observed in the case of infected sample. Several minor bands pertaining to host proteins were also observed in the preparation (Fig. 3a). In EBIA the cross adsorbed homologus antiserum raised against BCMV reacted positively with the 34 kDa band and none of protein bands from healthy showed positive reaction (Fig. 3b).

Coat protein gene amplification, sequencing and sequence analyses

In RT-PCR assay, a ~850bp product amplified using specific primers (AIB90/AIB91) corresponding to NIb/CP genes of BCMV was cloned and sequenced. None of other primer pairs used (Table 2) gave positive amplification. The sequenced NIb/CP fragment of the virus isolate contained 850 bases of which the first 27 bases correspond to the 3' end of NIb region while the remaining 823 bases correspond to the coat protein gene. The sequence was deposited in GenBank under the accession number EJ712783. In general the full length coat protein gene of BCMV consists of 860 nucleotides coding for 286 amino acids. Thus the present sequence lacks 37 bases coding for 12 amino acids at the C-terminal region of the coat protein. The partial CP gene contained 823 nucleotides which could potentially encode 274 amino acids. The partial CP sequence of the present isolate was compared with corresponding CP region sequences of a few representative isolate/strains of BCMV from India and other parts of the world (Table 3). BCMV sequence infecting vanilla in Madagascar, Reunion and French Polynesia were not included in the comparison as they contained only 327 bp sequence corresponding to the conserved core region of CP. Nucleotide sequence of present isolate of BCMV infecting vanilla (VP) shared 87-96% nucleotide and 87-98% amino acid identities with other BCMV isolate/strains taken for comparison. The present isolate showed highest nucleotide sequence identity of 96% with Blackeye (Blk1) and Florida (Fl) isolate/strains of BCMV while a maximum deduced amino acid sequence identity of 98% was observed with Blackeye (Blk1) and CAMV isolate/strains of BCMV.

Multiple sequence alignment of deduced amino acid sequence revealed a highly variable N-terminus region that had maximum sequence heterogenicity (up to first 50 amino acid residues) while the core region was found to be much conserved

Table 3: Source of BCMV partial CP gene used for sequence analysis

GenBank Accession No.	Isolate/strain	Designation used	Host	Country
Y11774	Peanut stripe	PStr1	Peanut	Australia
DQ897639	Siratro	Sir	Siratro	Brazil
AJ293276	Cowpea	Cowpea	Cowpea	China
DQ666332	NL4	NL4	*P. vulgaris*	Columbia
EU492546	HP	HP	Common bean	India
EU713858	NL-1	NL-1	*P. vulgaris*	India
FJ157246	K2	K2	Common bean	India
Z21700	370	370	Blotch isolate	Indonesia
AB012663	H	H	-	Japan
S66253	Blackeye	Blk3	-	Netherlands
AM258976	Peru	Peru	Lima bean	Peru
Z15057	Serotype B2	B2	-	Spain
AF395678	Blackeye	Blk1	-	Taiwan
CAU72204	CAMV	CAMV	Yardlong bean	Thailand
AF191748	Peanut stripe	PStr2	*Arachis hypogeae*	Thailand
Y17823	Florida	Fl	Cowpea	USA
U37072	US10	US10	*P. vulgaris*	USA
U23564	Dendrobium	Dend	*Dendrobium superbum*	USA
DQ925423	VN/BB2-6	Blk2	Black bean	Vietnam
AY112735	NL1	NL1	*P. vulgaris*	-
AF083559	NY15	NY15	-	-
AB270705*	N	PVY	Potato	Syria

**Potato virus Y* (PVY) taken as outgroup in phylogenetic studies

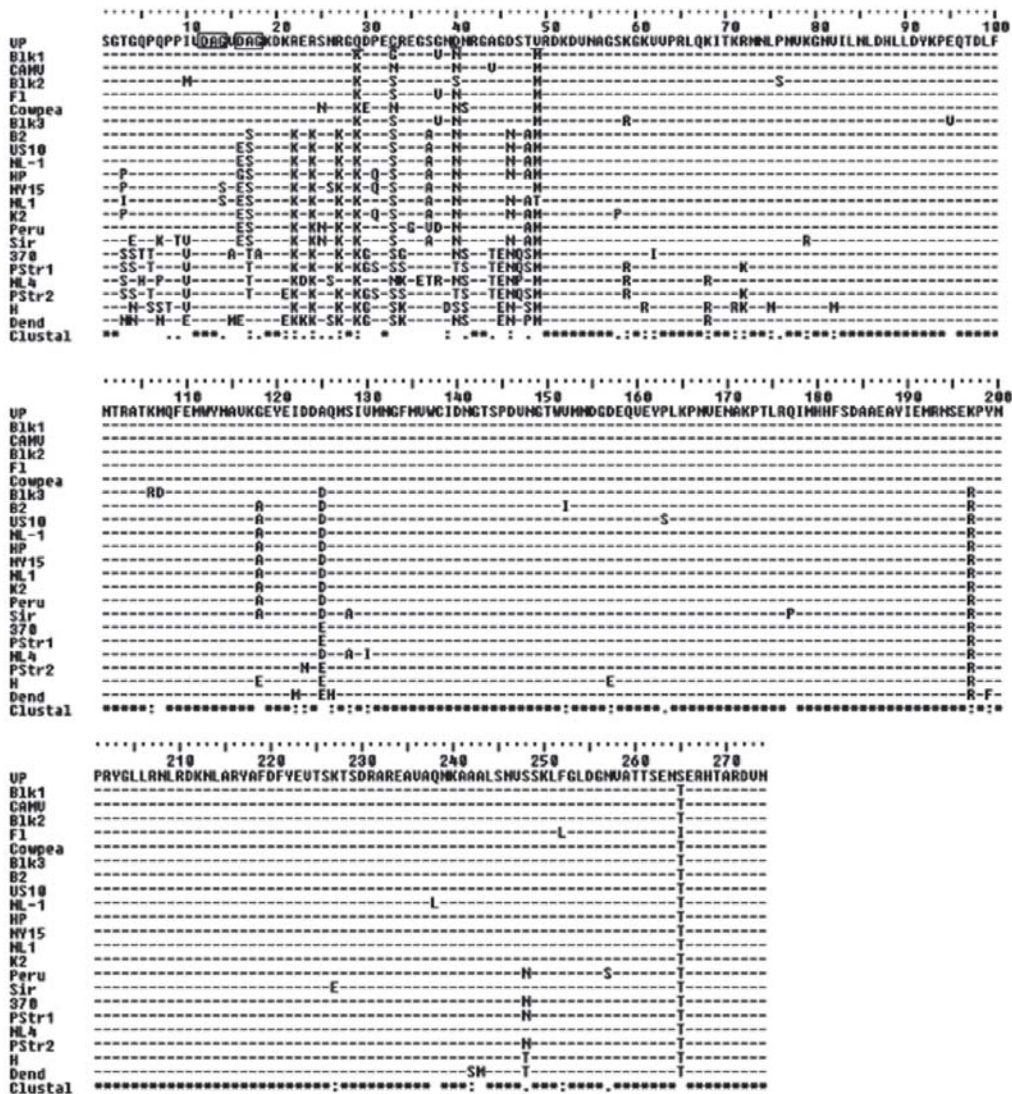

Fig. 4: *Multiple sequence alignment of deduced partial coat protein amino acid sequences of BCMV infecting V. planifolia (VP) in India with other isolate/strains of BCMV using Clustal X. Asterisk (*) indicates identity at a given position. DAG motifs are seen in box. Amino acids of the present sequence that differed from the others are underlined. Sequences for comparisons were obtained from GenBank and are shown in Fig. 5.*

(Fig. 4). Deduced amino acid sequence contained two 'DAG' motifs at positions 12-14 and 16-18. The sequence also contained two other motifs, 'MVWCIDN' at positions 135-141 and 'QMKAAA' at positions 238-243. These two motifs were found to be conserved in all the BCMV isolate/strains under comparison. Phylogram based on deduced partial amino acid sequence (Fig. 5) revealed three major clusters. The present isolate infecting vanilla in India (VP) formed a distinct cluster along with isolate/strains Blk1, Blk2, Blk3, Cowpea, CAMV and Fl while the isolate/strains infecting common bean in India (HP, NL-1 and K2) were placed in a different cluster. Based on the phylogram it is clear that clustering of the present BCMV isolate with other strain/isolates was not based on host or geographic location.

DISCUSSION

So far seven potyviruses *viz. Bean common mosaic virus* (BCMV), *Cowpea aphid borne mosaic virus* (CABMV), *Dasheen mosaic virus* (DsMV), *Watermelon mosaic virus* (WMV), *Wisteria vein mosaic virus* (WVMV), *Bean yellow mosaic virus* (BYMV) and *Ornithogalum mosaic virus* (OrMV) were reported to infect vanilla world wide (Grisoni *et al*., 2004, 2006). The present study showed the occurrence and identification of BCMV infecting vanilla in India on the basis of biological and CP sequence similarities. The virus isolate was found to be close to Blackeye cowpea strain of BCMV (BCMV-BlCpMV). Host range studies on 19 different plant species belonging to five families showed that the virus could infect *C. amaranticolor* belonging to Chenopodiaceae; *N. benthamiana*

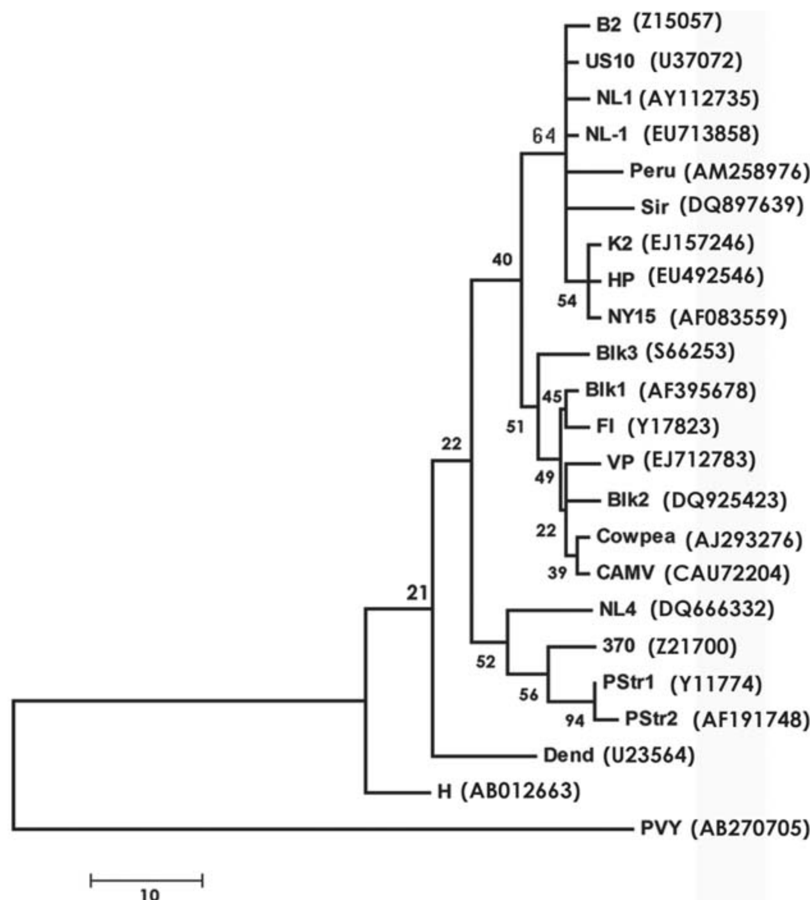

Fig. 5: *Phylogram, drawn by Maximum Parsimony Bootstrap method in MEGA (4.1), illustrating phylogenetic relationships based on the multiple alignments of the coat protein amino acid sequences of 21 distinct isolate/strains of BCMV and vanilla isolate of BCMV (VP). Potato virus Y (PVY) was taken as outgroup. Sequences for comparisons were obtained from GenBank and accession numbers are shown in parathesis. The boot strap values are shown at the nodis.*

from Solanaceae and *V. planifolia* from Orchidaceae. Of the nine species tested in Fabaceae, only *V. unguiculata* (cvs. C-52, C-152, Kanakamani and Lola) was found to be susceptible. The virus failed to infect any of the *Cucurbitaceae* members tested. BCMV isolates (WP010 and WP017) infecting *V. tahitensis* from French Polynesia induced systemic symptoms on *P. vulgaris* cv. Black Turtle 2 but failed to infect *V. unguiculata*, *V. marina* and *Macroptilum lathyrodoides* (Grisoni *et al*., 2004). However, the present BCMV isolate from *V. planifolia* did not infect *P. vulgaris*. The present virus induced blisters, mosaic, leaf distortion and vein banding on *Vigna unguiculata* (cv. C-52, C-152, Kanakamani) and mild mosaic on cv. Lola. Green vein banding, mosaic on various cultivars of *V. unguiculata* by Blackeye cowpea mosaic strain (BlCMV-FS, Florida isolate) of BCMV that was unable to infect any of the cultivars of *P. vulgaris* was reported (Murphy *et al*., 1987).

The first definitive evidence of BCMV infecting vanilla (*V. tahitensis*) was reported by Grisoni *et al.* (2004) based on sequence relationships of a 327 bp nucleotide fragment of the core region of coat protein gene. These BCMV vanilla isolates from French Polynesia shared 90.5-94.8% nucleotide identities with Azuki bean mosaic virus (ABMV) and Dendrobium mosaic virus (DeMV) strains of BCMV, respectively. Based on core region of CP, BCMV infecting *V. planifolia* were at least 82.5-88.3% identical to other BCMV isolates. However, so far full length coat protein gene sequence or sequence pertaining to the N-terminus of the coat protein of BCMV infecting vanilla is lacking. Further the validity of pairwise comparison of sequences of core region of coat protein of different potyvirus species as criterion for species discrimination within the family Potyviridae was questioned (Bos, 1992; Zettler, 1992). The deduced amino acid sequence of the coat protein of this present BCMV shared maximum sequence identity (98%) with Blackeye (Blk1) and CAMV isolate/strains of BCMV and thus indicated closeness to these cowpea isolates. This was further corroborated by the host range studies of the present isolate which easily infected different cultivars of cowpea. So far full coat protein gene sequence of cowpea strains of BCMV from India is not attempted. Hence in order to know the origin of the present isolate, more BCMV isolates from vanilla and cowpea from India need to be taken up. The present sequence is the largest BCMV isolate sequence available so far which is known to infect vanilla

and also the first record of BCMV on *V. planifolia* in India. As vanilla is vegetatively propagated through stem cuttings, RT-PCR method described can be used in nursery certification programs to identify virus-free planting material for further propagation and planting.

ACKNOWLEDGEMENTS

The authors are thankful to the Head (Crop Protection), Distributed information sub-centre (DISC) and Director, Indian Institute of Spices Research, Calicut, India for providing necessary facilities. We also thank, Incharge, Unit of Virology, Indian Agricultural Research Institute, New Delhi for assistance in electron microscopy work.

REFERENCES

Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W and Lipman, D.J. 1997. Gapped BLAST and PSI BLAST: a new generation of protein database search Programs. *Nucleic Acids Res*. **25**: 3389-3402.

Benson, D.A., Boguski, M.S.D., Lipman, J., Ostell, J., Ouellette, B.F., Rapp, B.A. and Wheeler, D.L. 1999. GenBank. *Nucleic Acids Res*. **27**: 12-17.

Berger, P.H., Wyatt, S.D., Shiel, P.J., Silbernagel, M.J., Druffel, K. and Mink, G.I. 1997. Phylogenetic analysis of Potyviridae with emphasis on legume-infecting potyviruses. *Arch. Virol*. **142**: 1979-1999.

Bhat, A.I., Bhadramurthy, V., Siju, S. and Hareesh, P.S. 2006. Detection and identification of *Cymbidium mosaic virus* infecting vanilla (*Vanilla planifolia* Andrews) in India based on coat protein gene sequence relationships. *J. Plant Biochem. & Biotech*. **15**: 33-37.

Bhat, A.I., Venugopal, M.N., Pant, R.P. and Bhai, R.S. 2004. Occurrence and distribution of viral diseases on vanilla (*Vanilla planifolia* Andrews) in India. *J. Spices & Aromatic Crops*. **13**: 143-148.

Bos, L., 1992. Potyviruses chaos or order? *Arch. Virol*. (Suppl). **5**: 31-46.

Clark, M.F., Lister, R.M. and Bar-Joseph, M. 1986. ELISA Techniques. *Methods Enzymol*. **118**: 742-766.

Drifhout, E. 1978. Genetic interactions between *Phaseolus vulgaris* L. and *Bean common mosaic virus* and its strains. Agricultural Research Report Pudoc (Wageningen).

Gough, K.H. and Shukla, D.D. 1981. Coat protein of Potyviruses. I. Comparison of the four Australian strains of Sugarcane mosaic virus. *Virology*. **11**: 455-462.

Grisoni, M., Davidson, F., Hyrondelle, C., Farreyrol, K., Caruana, M.L. and Pearson, M. 2004. Nature, incidence and symptomatology of viruses infecting *Vanilla tahitensis* in French Polynesia. *Plant Dis*. **88**: 119-124.

Grisoni, M., Moles, M., Farreyrol, K., Rassaby, L., Davis, R. and Pearson, M. 2006. Identification of potyviruses infecting vanilla by direct sequencing of a short RT-PCR amplicon. *Plant Pathol*. **55**: 523-529.

Khan, J.A., Lohuis, D., Goldbach, R. and Dijkstra, J. 1993. Sequence data to settle the taxonomic position of *Bean common mosaic virus* and *Blackeye cowpea mosaic virus* isolates. *J.Gen.Virol*. **74**: 2243-2249.

Laemmli, U.K., 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*. **227**: 680-685.

Madhubala, R., Bhadramurthy, V., Bhat, A.I., Hareesh, P.S., Retheesh, S.T. and Bhai, R.S. 2005. Occurrence of *Cucumber mosaic virus* on vanilla (*Vanilla planifolia* Andrews) in India. *J. Biosci*. **30**: 339-350.

Mink, G.I., Vetten, H.J., Ward, C.W., Berger, P.H., Morales, F.J., Myers, J.M., Silbernagel, M.J. and Barnett, O.W. 1994. Taxonomy and classification of legume-infecting potyviruses. A proposal from the Potyviridae Study Group of Plant Virus Sub-committee of ICTV. *Arch. Virol*. **139**: 231-235.

Murphy, J.F., Barnett, O.W. and Witcher, W. 1987. Characterisation of Blackeye cowpea mosaic virus strain from South Carolina. *Plant Dis*. **71**: 243-248.

O'Donell, I.J, Shukla, D.D. and Gough, K.H. 1982. Electroblot immunoassay of virus infected plant sap-A powerful technique for detecting plant viruses. *J. Virol. Methods*. **4**: 19-26.

Pappu, S.S., Brand, R., Pappu, H.R., Rybicki, E., Gough, K.H., Frenkel, M.J. and Niblett, C.L. 1993. A polymerase chain reaction method adapted for selective cloning of 3′ non translated regions of potyviruses: application to Dasheen mosaic virus. *J. Virol. Methods*. **41**: 9-20.

Rhoads, D.D. and Roufa, D.S. 1985. Emetine resistance of Chinese hamster cells: structure of wild type and mutant ribosomal protein S14 messenger RNA species. *Mol. Cell. Biol*. **5**: 1655-1659.

Siju, S., Madhubala, R. and Bhat, A.I. 2007. Sodium sulphite enhances RNA isolation and sensitivity of *Cucumber mosaic virus* detection by RT-PCR in black pepper. *J. Virol. Methods*. **141**: 107-110.

Vetten, H.J., Lesemann, D.E. and Maiss, E. 1992. Serotype A and B strains of Bean common mosaic virus are two distinct potyviruses. *Arch. Virol*. (Suppl) **5**: 415-431.

Zettler, F.W. 1992. Designation of potyvirus genera: a question of perspective and timing. *Arch. Virol*. (Suppl) **5**: 235-237.