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Sequence analysis and genetic diversity of five new Indian isolates of cucumber mosaic virus

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Summary. – Cucumber mosaic virus (CMV) is an important virus since it causes severe losses to many economically important crops worldwide. Five new isolates of CMV were isolated from naturally infected *Hippeastrum hybridum*, *Dahlia pinnata*, *Hemerocallis fulva*, *Acorus calamus* and *Typhonium trilobatum* plants, all exhibiting severe leaf mosaic symptoms. For molecular identification and sequence analyses, the complete coat protein (CP) gene of these isolates was amplified by RT-PCR. The resulting amplicons were cloned and sequenced and isolates were designated as HH (KP698590), DP (JF682239), HF (KP698589), AC (KP698588) and TT (JX570732). For study of genetic diversity among these isolates, the sequence data were analysed by BLASTn, multiple alignment and generating phylogenetic trees along with the respective sequences of other CMV isolates available in GenBank Database were done. The isolates under study showed 82–99% sequence diversity among them at nucleotide and amino acid levels; however they showed close relationships with CMV isolates of subgroup IB. In alignment analysis of amino acid sequences of HH and AC isolates, we have found fifteen and twelve unique substitutions, compared to HF, DP and TT isolates, suggesting the cause of high genetic diversity.

Keywords: cucumber mosaic virus; new isolates; capsid protein; genetic diversity

Introduction

Cucumber mosaic virus (CMV) has been reported to infect more than 1200 monocot or dicot plant species of different families and has worldwide distribution in almost every climatic zone (Jacquemond, 2012). CMV is the prototype species of the genus *Cucumovirus* of the family *Bromoviridae* which also includes two other members: peanut stunt virus (PSV) and tomato aspermy virus (TAV) within the genus. CMV is mainly transmitted by aphids under natural conditions and by mechanical inoculations to other plants. The genome of CMV consists of three single-stranded positive-sense RNA species designated as RNA1, RNA2, and RNA3, according to their molecular weight. The strains of CMV have been divided into two subgroups (I and II) based on serological properties, nucleic acid hybridization, nucleic acid and/or protein sequence composition, RNase protection assay and RT-PCR RFLP analysis. Further splitting of subgroup I into IA and IB has been proposed on the basis of sequence data, analysis of 5'-non-translated region of RNA3 of several strains and phylogenetic analysis of CP (Roossinck *et al*., 1999) and accordingly the Asian strains including the Indian ones have been grouped into the subgroup IB (Palukaitis and Garcia-Arenal, 2003).

Dahlia (*Dahlia pinnata*), amaryllis (*Hippeastrum hybridum*) and day lily (*Hemerocallis fulva*) are popular ornamental plants. They are grown in garden beds and pots for their beautiful flowers of various colours and also used as cut flowers because of their long vase life. While, typhonia (*Typhonium trilobatum*) is an aroid plant distributed throughout India. It has many medicinal uses and its tubers are consumed by some Indian tribal societies (http://www.flowersofindia.net/catalog/slides/Bengal%20Arum.html).

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Abbreviations: CMV = cucumber mosaic virus; CMV-HH = *Hippeastrum hybridum* isolate; -DP = *Dahlia pinnata* isolate; -HF = *Hemerocallis fulva* isolate; -AC = *Acorus calamus* isolate; -TT = *Typhonium trilobatum* isolate; PSV = peanut stunt virus; TAV = tomato aspermy virus

The sweet flag (*Acorus calamus*) is a perennial herb. It has been used medicinally for a wide variety of ailments, and its aroma makes calamus essential oil valued in the perfume industry (http://en.wikipedia.org/wiki/Acorus_calamus).

In present communication, the natural occurrence of CMV on *H. hybridum*, *D. pinnata*, *H. fulva*, *A. calamus* and *T. trilobatum* plant species is being reported for the first time from India. Molecular analysis of coat protein gene of these CMV isolates has been done to study the genetic diversity among these isolates which suggested high sequence diversity among them.

Materials and Methods

Samples and plant infection. The naturally infected plants of *D. pinnata*, *H. hybridum*, *H. fulva*, *A. calamus* and *T. trilobatum* showing virus like symptoms were collected from gardens and nurseries of Lucknow and maintained in an insect-proof greenhouse at NBRI, Lucknow for further study. For virus transmission studies, the test species of *Amaranthus tricolor*, *Chenopodium amaranticolor*, *C. album*, *Cucumis sativus*, *Datura metel*, *Lycopersicon esculentum*, *Spinacia oleracea*, *Nicotiana tabacum* cv. 'White Burley', *N. rustica*, *N. glutinosa*, *N. benthamiana* and *Petunia hybrida* were inoculated separately using the sap of young leaf tissues from each symptomatic plants macerated independently in inoculation buffer in a ratio of 1:10 (w/v) as described by Noordam (1973). The inoculated plants were observed for 30 days for symptoms.

Cloning of cucumovirus CP gene. For molecular identification of virus, the total RNA was extracted from 100 mg of fresh leaf tissue of symptomatic plants ground in liquid nitrogen using TRIzol reagent (Sigma, USA) and reverse transcription-PCR (RT-PCR) was performed using cucumovirus specific primers as described earlier (Kumar *et al.*, 2014). The amplified products were analysed by electrophoresis on 1% agarose gel and size was assessed by 1 kb DNA ladder (Genei Pvt. Ltd, India). The amplified products were gel purified using QIAquick gel extraction kit (Qiagen GmbH, Germany) and ligated into pGEM-T Easy vector system-I (Promega, USA). The chemically competent *E. coli* DH5α cells were transformed. The clones were screened by digestion with *Eco*RI restriction enzyme and three positive clones from each isolate were sequenced.

Sequence analysis. Sequence data obtained were analyzed and consensus sequence of three clones of each isolate were determined and deposited in the GenBank Database. The open reading frame (ORF) was predicted by ORF finder program (http://www.ncbi.nlm.nih.gov/projects/gorf/) to find in frame AUG-start and TAG-

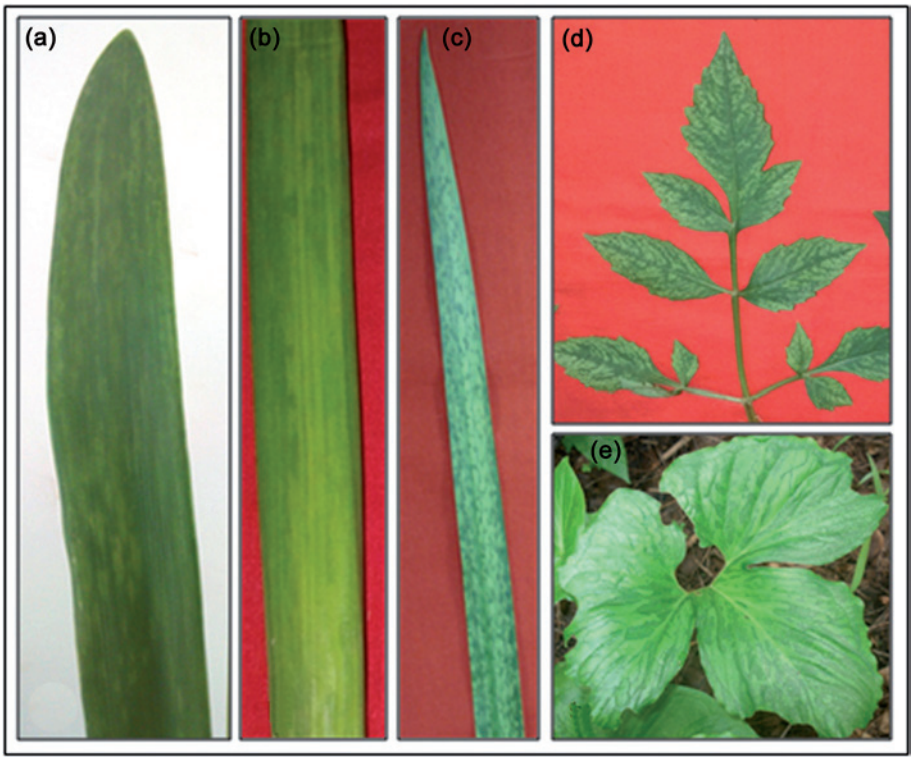

Fig. 1

Symptoms observed on different plants

Severe mosaic and leaf stripe symptoms observed on naturally infected *Hippeastrum hybridum* (a), *Hemerocallis fulva* (b), *Acorus calamus* (c), *Dahlia pinnata* (d) and *Typhonium trilobatum* (e) plants growing in gardens at Lucknow.

termination codons and translated into putative amino acids using the *ExPasy* tool (http://www.expasy.org/tools/dna.html). To observe the sequence identity of virus isolates under study, the sequence data were initially analyzed by BLASTn (http://www.ncbi.nlm.nih.gov/Blast.cgi). Further, the sequence identity among them and with selected CMV isolates of subgroup IA, IB and II group members were determined by *Genomatix DiAlign* 2.1 program (http://www.genomatix.de/cgi-bin/dialign/dialign.pl). For phylogenetic relationships the phylogenetic analysis was performed using MEGA v6.1 program (Tamura *et al*., 2013). The phylogram was generated with 1,000 bootstrap replicates and peanut stunt virus and tomato aspermy virus were used as reference out group sequences of the *Cucumovirus* genus for the rooting of tree.

Results

The natural symptoms of dark green stripes on leaves of *H. hybridum* (Fig. 1a); mottling on *D. pinnata* (Fig. 1d); mosaic on *T. trilobatum* (Fig. 1e); yellow stripes on leaves of *H. fulva* (Fig. 1b) and severe dark green mosaic on leaves of *A. calamus* (Fig. 1c) were observed during the survey in and around Lucknow. The inoculations of leaf saps from *H. hybridum*, *D. pinata*, *H. fulva*, *A. calamus* and *T. trilobatum* induced chlorotic/necrotic local lesions on *C. album*, *C. amaranticolor* and *S. oleracea* plants 7 days post infection. The sap inoculations of *H. hybridum*, *D. pinnata* and *H. fulva* induced more or less similar systemic mosaic symptoms 25 days post infection. While, sap inoculations of *H. hybridum* and *A. calamus* induced lethal necrosis leading to death in later stages of inoculated plants. All the five isolates under study induced mosaic symptoms on *C. sativus* which are similar to those induced by CMV reported earlier (Francki *et al*., 1979).

During analysis of RT-PCR products, the expected size amplicons of ~650 bp were obtained by CMV specific primers from naturally infected samples of *H. hybridum*, *D. pinnata*, *H. fulva*, *A. calamus* and *T. trilobatum* which were similar to a CMV infected *N. tabaccum* sample taken as a positive control (Kumar *et al*., 2014) but not in healthy

Table 1. Cucumber mosaic virus (CMV) isolates along with CMV isolates of subgroup IA, IB and II and the other two members of genus cucumovirus: peanut stunt virus (PSV) and tomato aspermy virus (TAV) used for this study

Acc. No.	Virus strain	Isolation host	Country	Subgroup
KP698590	CMV-HH*	*Hippeastrum hybridum*	India	Under study
KP698589	CMV-HF*	*Hemerocallis fulva*	India	Under study
JF682239	CMV-DH*	*Dahlia pinnata*	India	Under study
KP698588	CMV-AC*	*Acorus calamus*	India	Under study
JX570732	CMV-TT*	*Typhonium trilobatum*	India	Under study
EF178298	CMV-Ban	*Musa paradisiaca*	India	IB
EF153733	CMV-Chry	*Chrysanthemum morifolium*	India	IB
EF153734	CMV-Ts	*Solanum lycopersicum*	India	IB
EF593023	CMV-A	*Amaranthus cruentus*	India	IB
HQ343232	CMV-Egg	*Solanum melongina*	India	IB
JN692495	CMV-Ger	*Gerbera jamesonii*	India	IB
JN642676	CMV-Pet	*Petunia hybrida*	India	IB
Y16926	CMV-Tfn	*Solanum lycopersicum*	Italy	IB
U20219	CMV-Ix	*Solanum lycopersicum*	USA	IB
AF127977	CMV-K	*Nicotiana spp.*	China	IB
KF891361	CMV-BoG1	*Lagenaria siceraria*	India	IB
AY690621	CMV-PL*	*Piper longum*	India	IB
JF279609	CMV- Bal-In	*Cucumis melo*	India	IB
D10538	CMV-Fny	*Cucurbita pepo*	USA	IA
D00385	CMV-O	–	Japan	IA
D12499	CMV-Y	–	Japan	IA
EU642567	CMV-Car*	*Daucus carota*	India	II
AJ866272	CMV-Gera1	*Pelargonium* spp.	India	II
L15336	CMV-Trk	*Trifolium repens*	Hungary	II
JN135292	PSV-ER	*Pisum sativum*	Poland	Out group
EU163411	TAV-Chry*	*Chrysanthemum morifolium*	India	Out group

(–) = information not available, (*) = strains abbreviated for this study.

Table 2. Sequence identity matrixes of HH isolate with HF, DP, AC and TT isolates along with other member of CMV subgroup IB, IA and II

	CMV subgroup IB isolates															IA isolates			II isolates			Out-group	
	HH	**DP**	**HF**	**AC**	**TT**	**BoG1**	**PL**	**Bal-In**	**Ban**	**Chry**	**Ts**	**A**	**Egg**	**Ger**	**Pet**	**Fny**	**O**	**Y**	**Car**	**Gera1**	**Trk**	**PSV**	**TAV**
HH	–	**90**	**83**	**90**	**90**	**92**	**93**	**92**	**91**	**87**	**92**	**88**	**90**	**88**	**90**	**91**	**90**	**90**	**73**	**76**	**75**	**45**	**37**
DP	89	–	**93**	**85**	**99**	**97**	**97**	**96**	**99**	**95**	**96**	**96**	**98**	**98**	**99**	**95**	**95**	**94**	**78**	**81**	**80**	**47**	**43**
HF	83	96	–	**79**	**93**	**90**	**90**	**89**	**92**	**88**	**90**	**89**	**91**	**91**	**92**	**89**	**89**	**88**	**73**	**75**	**74**	**44**	**42**
AC	94	88	82	–	**85**	**87**	**87**	**86**	**86**	**82**	**86**	**83**	**85**	**83**	**86**	**85**	**85**	**84**	**69**	**72**	**71**	**40**	**34**
TT	89	99	96	88	–	**96**	**97**	**95**	**99**	**95**	**96**	**96**	**97**	**97**	**99**	**95**	**95**	**94**	**78**	**81**	**80**	**46**	**43**
BoG1	93	94	90	91	94	–	**99**	**98**	**97**	**94**	**98**	**94**	**97**	**95**	**97**	**97**	**97**	**96**	**78**	**82**	**80**	**48**	**42**
PL	93	94	88	91	94	98	–	**98**	**98**	**94**	**99**	**95**	**97**	**95**	**97**	**98**	**97**	**97**	**78**	**82**	**80**	**48**	**42**
Bal-In	92	93	89	92	93	96	96	–	**96**	**93**	**97**	**94**	**95**	**94**	**96**	**97**	**96**	**95**	**78**	**81**	**79**	**48**	**41**
Ban	89	99	96	88	99	94	94	93	–	**95**	**97**	**96**	**98**	**97**	**99**	**96**	**96**	**95**	**78**	**82**	**80**	**46**	**43**
Chry	87	95	91	88	95	92	92	90	95	–	**93**	**99**	**94**	**94**	**94**	**93**	**92**	**92**	**75**	**78**	**77**	**47**	**40**
Ts	88	91	88	88	91	93	92	92	91	90	–	**94**	**96**	**94**	**96**	**99**	**98**	**97**	**78**	**82**	**80**	**45**	**41**
A	87	95	92	88	95	92	93	90	95	99	90	–	**95**	**94**	**95**	**94**	**93**	**93**	**76**	**79**	**77**	**47**	**41**
Egg	88	97	93	87	97	92	93	92	97	92	90	94	–	**96**	**98**	**95**	**95**	**94**	**78**	**81**	**80**	**46**	**43**
Ger	88	98	95	87	98	93	93	92	98	94	91	94	96	–	**97**	**94**	**94**	**93**	**77**	**80**	**79**	**49**	**43**
Pet	89	97	94	89	97	94	93	93	98	94	91	95	98	96	–	**95**	**95**	**94**	**78**	**82**	**80**	**46**	**42**
Fny	88	91	85	85	90	91	91	92	91	89	94	89	89	90	91	–	**98**	**97**	**79**	**82**	**80**	**45**	**42**
O	87	89	84	87	89	92	91	92	90	89	94	89	87	89	89	98	–	**96**	**79**	**82**	**81**	**45**	**41**
Y	87	89	85	87	89	91	91	91	89	89	94	90	87	88	89	97	96	–	**78**	**79**	**80**	**45**	**41**
Car	68	68	64	67	68	69	68	69	69	69	68	69	66	67	69	68	68	67	–	**93**	**91**	**50**	**42**
Gera1	71	72	70	71	72	73	72	70	73	71	73	71	72	71	73	70	71	71	95	–	**96**	**51**	**44**
Trk	70	69	66	71	69	72	72	72	69	70	70	70	68	68	69	73	71	70	95	97	–	**50**	**43**
PSV	41	41	39	42	41	42	41	45	43	41	44	41	41	41	41	44	42	42	40	42	42	–	**66**
TAV	35	37	35	35	36	38	38	35	37	35	38	35	38	39	35	36	34	38	41	40	38	59	–

Values above the diagonal (in bold) represent amino acid sequence identities whereas below the diagonal represent nucleotide sequence identities. Highest identity values have been highlighted with grey shading. Details of strains used in this study have been given in Table 1.

samples collected from the same location. The similar size (~650 bp) amplification product was also observed in experimentally inoculated plants, suggesting successful transmission of the virus isolates.

The ~650 bp amplicons obtained from *H. hybridum*, *D. pinnata*, *H. fulva*, *A. calamus* and *T. trilobatum* samples were cloned into pGEM-T Easy vector system-I and white colonies were screened by digestion with *Eco*RI which resulted in successful integration of CP transgene. Three clones for each of five samples were sequenced and the data obtained were analyzed to eliminate any ambiguity. The sequence data of consensus 657 bp sequence from each sample, translating the complete CP gene, was deposited in GenBank Acc. Nos. KP698590, JF682239, KP698589, KP698588 and JX570732 and designated as HH, DP, HF, AC and TT isolates, respectively.

BLASTn analysis of HH isolate (KP698590) revealed 93–94% nucleotide sequence identities with various isolates of CMV: BoG1 (KF891361), Betel vine (AY690620), Amaryllis (EF187825), SG1 (KJ874248), Ker (JX112021), Esf172 (JX025995), Bas3 (JX025989), Banana (AY125575), and AjS4 (JX025999) reported from India and Iran. The HF isolate (KP698589) showed 95–96% identities with CMV isolates of Gerbera (JX913531, JN692495, JF682237, JX888093), Banana (DQ910858), Eggplant (GU906293), Petunia (JF798578), Salvia (EU600215) and ZM5 (KJ746023) reported from India and China. The DP isolate (JF682239) showed 98–99% identities with CMV isolates of Gerbera (JN692495, JF682237, JX888093), Banana (DQ910858), Petunia, Salvia, ZM5, SB2 (KJ746019), SB3 (KJ746020), CLW2 (JN054635), C18 (EU310928) and XD4 (KJ746015) reported from India, Malaysia and China. The AC isolate (KP698588) showed 92-95% identities with CMV isolate of Banana, BoG1, Esf172, Bas3, SG1, AjS4 and Vir56 (DQ006805) reported from India and Italy. The TT isolate (JX570732) showed 98–99% identities with CMV isolates of Gerbera and Banana, Petunia, Salvia, ZM5, SB2 and SB3 reported from India and China. Based on 94–99% sequence identities, the virus isolates infecting *H. hybridum*, *H. fulva*, *D. pinnata*, *A. calamus* and *T. Trilobatum* were identified as new isolates of CMV and designated as CMV-HH, -HF, -DP, -AC, and -TT isolates, respectively.

To obtain the genetic diversity among CMV-HH, -HF, -TT, -AC and -DP isolates under study, the nucleotide and

amino acid sequences of these isolates were aligned with the respective sequence of selected CMV isolates of subgroup IA, IB and II (Table 1). The analysis revealed high sequence diversity among them and with other CMV members of subgroup IA, IB and II (Table 2). The isolates under study shared 82-99% nucleotide and 83–99% amino acid identities to each other. Among the five isolates, HH and AC isolates were found to be highly diverged as compared to HF, DP and TT isolates suggesting genetic differences in their genome.

When the CP amino acid sequences of the CMV isolates under study were multiple aligned with a reference sequence of subgroup IB (Ger), IA (Fny) and II (Trk), the HH isolate showed fifteen unique amino acid substitutions: V20G, T21S, P26S, I112N, F136V, K145D, R146G, P147A, G150V, S154Q, I157A, T163N, T165K, C188Y and I193A scattered throughout the CP region, however the helix and β-sheets were unaffected (Fig. 2). The AC isolate also showed twelve unique amino acid substitutions: V20G, T21S, I112N, F136V, K145D, R146G, P147A, G150V, S154Q, I157A, C188Y and I193A which were commonly shared by HH isolate. Whereas HF, DP and TT isolates did not show any substitutions.

To infer the phylogenetic relationships of HH, HF, TT, AC and DP isolates with selected CMV isolates of subgroup IA, IB and II members (Table 1), NJ trees were generated employing nucleotide and amino acid sequences of respective gene (Fig. 3a; 3b) which showed a clear cut clustering with CMV subgroup IA, IB and II members. The HH and AC isolates showed distant relationships with CMV isolates (Ger, Ban, Egg, Pet, Chry and A) of subgroup IB reported from Northern part of India, whereas close relationships with CMV isolates (BoG1, PL and Bal-In), reported from Southern India at both nucleotide and amino acid levels. They showed some affinity to subgroup IA members including the CMV-Ts isolate infecting tomato in Northern India (Pratap *et al.*, 2012) that has been reported to show the affinity with CMV isolates of subgroup IA. While, the other three CMV isolates: HF, TT and DP showed close relationships with CMV subgroup IB isolates (Ger, Ban, Egg, Pet, Chry and A) reported from Northern part of India, both at

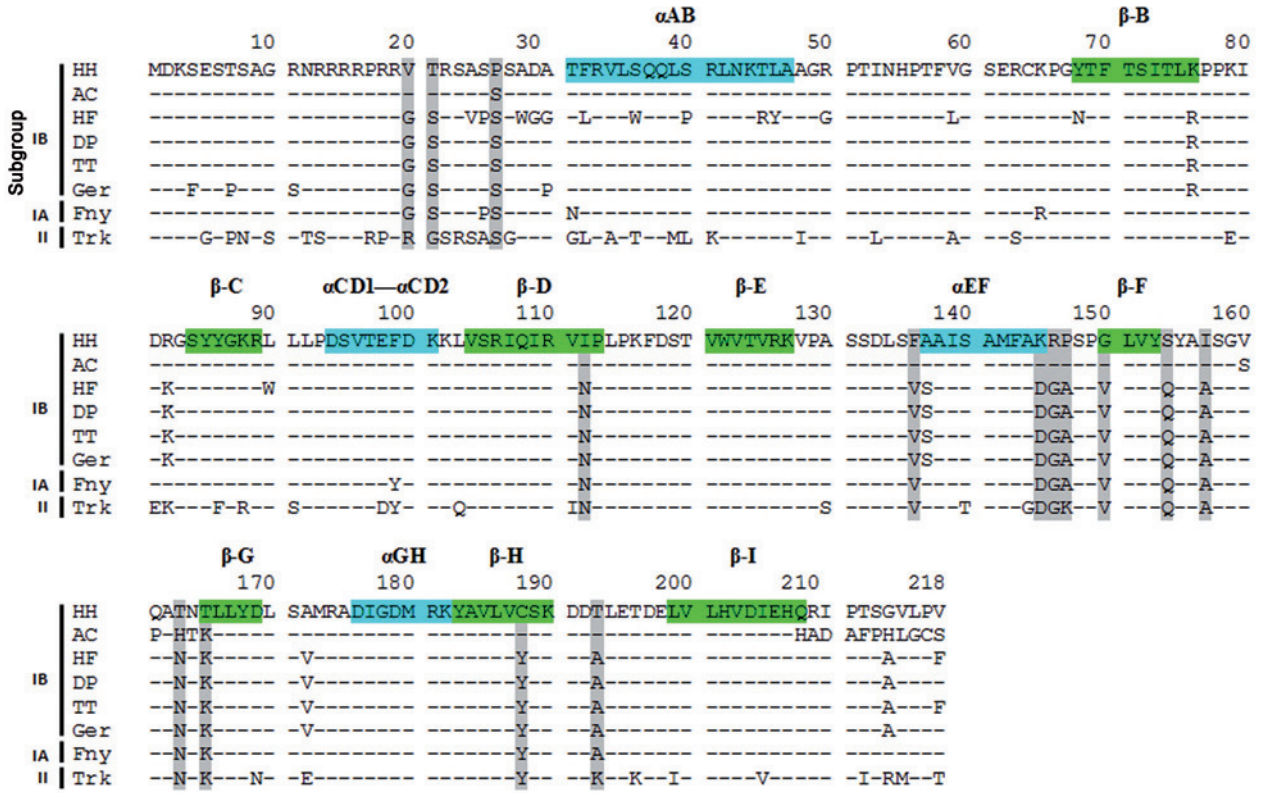

Fig . 2

Multiple alignment analysis of capsid protein amino acid sequence of CMV-HH, -AC, -HF, -DP and -TT isolates

Reference sequences of subgroup IB, IA and II showing the conserved helix (highlighted in turquoise) and β-sheet (highlighted in green) regions and unique amino acid substitutions. The grey regions are helices and green are β-strands. The grey shadowed amino acids are unique amino acid substitutions in CMV-HH isolate.

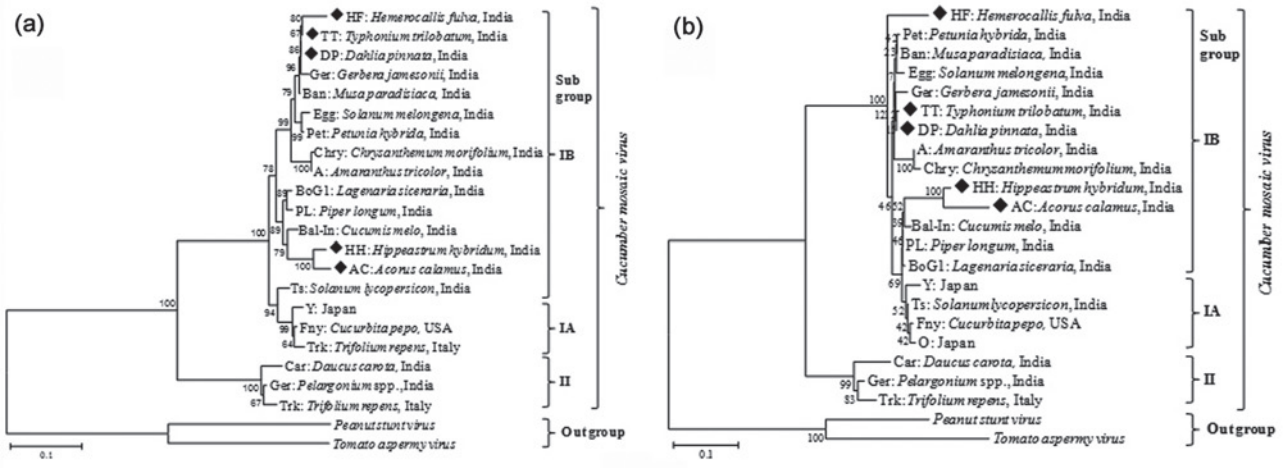

Fig. 3

Phylogenetic analysis of nucleotide sequences (a) and amino acid sequences (b) of CMV-HH, -AC, -HF, -DP and -TT isolates
Close members of CMV strains, along with reference members of subgroup IB, IA and II (Table 1) reported worldwide are included. Evolutionary history was inferred using the Neighbor-joining method conducted in MEGA6. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches.

nucleotide (Fig. 3a) and amino acid (Fig. 3b) levels. These analyses suggested high genetic diversity among the isolates under study.

Discussion

In present study, five new host species (*H. hybridum*, *D. pinnata*, *H. fulva*, *A. calamus* and *T. trilobatum*) of CMV has been identified from India based on virus transmission by sap, RT-PCR detection using CMV specific primers and sequence analyses of PCR amplicon. The transmission of the virus isolates through sap and induction of systemic mosaic on *C. sativus* (a diagnostic host of CMV, Francki *et al*., 1979) indicated the association of CMV. Initially CMV was successfully detected from all infected plant samples by RT-PCR using cucumovirus genus specific primers (Kumar *et al*., 2014) which revealed the successful amplification of ~650 bp band suggesting the infection of CMV in these plant samples.

The literature revealed the reports of potyvirus on amaryllis from India (Raj *et al*., 2009), tomato mosaic virus on *H. fulva* from Brazil (Duarte *et al*., 2007) and dasheen mosaic potyvirus on *T. trilobatum* from Vietnam (Ha *et al*., 2008). However, infection of CMV on *A. calamus*, *D. pinnata*, *T. trilobatum* and *H. fulva* has not been reported from any part of the world. In present study, molecular identification of CMV subgroup IB isolates infecting five plant species (*H. hybridum*, *D. pinata*, *H. fulva*, *A. calamus* and *T. trilobatum*) is being reported.

Earlier studies suggested that sequence data of *CP* gene of CMV is sufficient for identification of virus isolate into proper taxonomic position (Srivastava and Raj, 2004a; Verma *et al*., 2005; Roossinck, 2002), therefore, only *CP* gene was amplified using its specific primers (Kumar *et al*., 2014). Sequence analysis of amplicons revealed presence of the similar 657 nucleotide length of *CP* gene in all five isolates (HH, HF, TT, AC and DP) as reported for all CMV isolates (Palukaitis and Garcia-Arenal, 2003). The alignment of nucleotide and amino acid residues of CP sequences revealed that these isolates shared high sequence diversity among them and with other CMV members of subgroup IB. Sequence analysis also revealed that the HH isolate showed fifteen unique amino acid substitutions throughout the CP region, of which twelve substitutions were also shared by AC isolate. Because of these common amino acid substitutions, HH and AC isolates clustered together in a cluster and exhibited comparatively low affinity to subgroup IB members similar as compared to the HF, DP and TT isolates which showed high affinity to the subgroup IB members similar as most of the Asian isolates (Srivastava *et al*., 2004b). The isolates under study showed 82–99% nucleotide and 83–99% amino acid identities to each other, of which HH and AC isolates had high sequence diversity (82–83%) as compared to HF, DP and TT isolates (which showed 97–99% diversity), suggesting genetic variations in their genome. HH isolate showed fifteen unique amino acid substitutions (scattered throughout the CP region) during multiple alignments with reference sequences of CMV isolates of subgroup IA (Fny, Owen *et al*., 1990), IB (Ger, Gautam *et al*., 2014) and II (Trk, Salanki *et*

al., 1994), of which twelve substitutions were shared by AC isolate, however, in both the cases helix and β-sheets seems to be least affected as they have crucial roles in folding and of configuration of CP (Smith *et al.*, 2000; Thomas *et al.*, 2000). These amino acid substitutions may have some impact on the coat protein orientation, symptoms expression, transmission as described earlier (Perry *et al.*, 1998; Wong *et al.*, 1999) which required further investigations. Based on these analyses, the HH, HF, TT, AC and DP isolates were identified as new isolates of CMV of subgroup IB that had high genetic diversity among them.

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References

Duarte LML, Alexandre MAV, Rivas EB, Cattai MB, Soares RM, Harakava R, Fernandes FMC (2007): Phylogenetic analysis of tomato mosaic virus from Hemerocallis sp. and Impatiens hawkeri. Summa Phytopathol. 33, 409–413. http://dx.doi.org/10.1590/S0100-54052007000400016

Gautam KK, Raj R, Kumar S, Raj SK, Roy RK, Katiyar R (2014): Complete sequence of RNA3 of Cucumber mosaic virus isolates infecting Gerbera jamesonii suggests its grouping under IB subgroup. Virus Dis. 25, 398–401. http://dx.doi.org/10.1007/s13337-014-0216-x

Ha C, Revill P, Harding RM, Vu M, Dale JL (2008): Identification and sequence analysis of potyviruses infecting crops in Vietnam. Arch. Virol. 153, 45–60. http://dx.doi.org/10.1007/s00705-007-1067-1

Jacquemond M (2012): Cucumber mosaic virus. Adv. Virus Res. 84, 439–504. http://dx.doi.org/10.1016/B978-0-12-394314-9.00013-0

Kumar S, Gautam KK, Raj SK (2014): Molecular identification of cucumber mosaic virus isolates of subgroup IB associated with mosaic disease of eggplant in India. Virus Dis. 25, 129–131. http://dx.doi.org/10.1007/s13337-013-0174-8

Noordam D (1973): Identification of plant viruses. Methods and experiments. New York, USA: Oxford and IBH Publishing Company.

Owen JE, Shintaku M, Aeschleman P, Tahar SB, Palukaitis P (1990): Nucleotide sequence and evolutionary relationship of Cucumber mosaic virus (CMV) strains: CMV RNA 3. J. Gen. Virol. 71, 2243–2249. http://dx.doi.org/10.1099/0022-1317-71-10-2243

Palukaitis P, Garcia-Arenal F (2003): Cucumoviruses. Adv. Virus Res. 62, 241–323. http://dx.doi.org/10.1016/S0065-3527-(03)62005-1

Perry KL, Zhang L, Palukaitis P (1998): Amino acid exchange in the coat protein of cucumber mosaic virus differentially affects transmission by the aphids: Myzus persicae and Aphis gossypii. Virology 242, 204–210. http://dx.doi.org/10.1006/viro.1998.8991

Pratap D, Kumar S, Snehi SK, Raj SK (2012): Biological and molecular characterization of cucumber mosaic virus isolate causing shoestring disease of tomato in India which has closer affinity to European or East Asian isolates of CMV. Indian J. Virol. 23, 57–63. http://dx.doi.org/10.1007/s13337-012-0059-2

Raj SK, Snehi SK, Kumar S, Khan MS (2009): First molecular detection and identification of a potyvirus associated with severe mosaic disease of amaryllis (Hippeastrum hybridum Hort.) in India. Aust. Plant Dis. Notes 4, 50–53.

Roossinck MJ (2002): Evolutionary history of cucumber mosaic virus deduced by phylogenetic analysis. J. Virol. 76, 3382–3387. http://dx.doi.org/10.1128/JVI.76.7.3382-3387.2002

Roossinck MJ, Zhang L, Hellwald KH (1999): Rearrangements in the 5' nontranslated region and phylogenetic analyses of cucumber mosaic virus RNA 3 indicate radial evolution of three subgroups. J.Virol. 73, 6752–6758.

Salanki K, Thole V, Balazs E, Burgyan J (1994): Complete nucleotide sequence of the RNA 3 from subgroup II of Cucumber mosaic virus (CMV) strain: Trk7. Virus Res. 31, 379–384. http://dx.doi.org/10.1016/0168-1702(94)90030-2

Smith TJ, Chase E, Schmidt T, Perry KL (2000): The structure of cucumber mosaic virus and comparison to Cowpea chlorotic mottle virus. J. Virol. 74, 7578–7586. http://dx.doi.org/10.1128/JVI.74.16.7578-7586.2000

Srivastava A, Chandra G, Raj SK (2004): Molecular characterization of a strain of Cucumber mosaic virus based on coat protein and movement protein genes. Acta Virol. 48, 229–239.

Srivastava A, Raj SK (2004): High molecular similarity among Indian isolate of cucumber mosaic virus suggests a common origin. Curr. Sci. 87, 1126–1131.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013): MEGA6: Molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. 30, 2725-2729. http://dx.doi.org/10.1093/molbev/mst197

Thomas SJ, Chase E, Schmidt T, Perry KL (2000): The structure of cucumber mosaic virus and comparison to Cowpea chlorotic mottle virus. J. Virol. 74, 7578–7586. http://dx.doi.org/10.1128/JVI.74.16.7578-7586.2000

Verma N, Singh L, Singh AK, Kulshrestha S, Raikhy G, Hallan V, Ram R, Zaidi AA (2005): Ornithogalum: a new host of Cucumber mosaic virus (CMV) from India. Plant Pathol. 54, 256. http://dx.doi.org/10.1111/j.1365-3059.2005.01128.x

Wong S, Thio SS, Shintaku MH, Palukaitis P (1999): The rate of cell-to-cell movement in squash of Cucumber mosaic virus is affected by sequences of the capsid protein. Mol. Plant-Microbe Interact. 12, 628–632. http://dx.doi.org/10.1094/MPMI.1999.12.7.628