MOLECULAR CHARACTERIZATION OF A CARNATION ETCHED RING VIRUS ISOLATE FROM INDIA

G. RAIKHY, V. HALLAN, S. KULSHRESTHA, M. LAL SHARMA, R. RAM, A.A. ZAIDI*

Plant Virology Laboratory, Floriculture Division, Institute of Himalayan Bioresource Technology, CSIR, Palampur, Himachal Pradesh, 176 061, India

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Summary. – Incidence of the Carnation etched ring virus (CERV), the only DNA virus reported to date on carnation, was investigated by a bioassay using a partially purified virus as inoculum and then by a double-antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA). Out of 61 carnation cultivars analyzed 41 (67%) were found positive. The virus positivity was verified by polymerase chain reaction (PCR) and nucleotide sequencing. The amplified 1349 bp fragment was by about 98% and 96% identical with respect to coat protein (CP) and enzymatic polyprotein genes, respectively, as compared to the sequences available in the database. In terms of amino acid sequence similarity, the homology values were 99% and 97%, respectively. Comparison with other caulimoviruses revealed that CERV is most closely related to the Cauliflower mosaic virus (CaMV). High genetic stability of CERV may be attributed to the fact that it has evolved from the same initial sequence in an original host. Because of global market of cut flowers and vegetative propagation it has been dispersed around the world.

Key words: carnation; Carnation etched ring virus; coat protein; enzymatic polyprotein; Indian isolate; nucleotide sequencing

Introduction

Carnation (*Dianthus caryophyllus*) is one of important cut flower crops worldwide. Although no official statistics is available, it is estimated that it is grown on total 6,000–7,000 ha worldwide (Pallas *et al*., 1999). Out of eleven viruses currently known to infect carnation in nature only six are economically important (Pallas *et al*., 1999). Among these viruses only CERV (the species *Carnation etched ring virus*, the genus *Caulimovirus*, the family *Caulimoviridae*) virion has a circular double-stranded DNA genome of approximately 8 kbp that is encapsidated in a spherical naked nucleocapsid of about 50 nm in diameter. Lommel *et al.* (1983) and Rodoni *et al*. (1994) have surveyed the spread of viral diseases in commercial carnation cultivars and have found that although the CERV infection was less incident it was second only to the Carnation mottle virus (CarMV). Symptoms of CERV infection consist of necrotic flecks, rings and line patterns "etched" on leaves. CERV has been reported from various parts of the world (Lisa, 1995) including France (Devergne, 1984), Italy (Rana, 1984) and Finland (Bremer and Lahdenpera, 1981). On the other hand, from Asia, there are very few reports only (Smookler and Loebenstein, 1975; Ram *et al*., 1998).

Although a complete nucleotide sequence of CERV has already been reported (Hull *et al*., 1986), there is no such a report from Asia.

In this study, an attempt to characterize a first CERV isolate from India was made; namely (i) its detection and incidence in naturally and commercially grown carnation cultivars by a bioassay, DAS-ELISA and PCR, and (ii) its comparison with other CERV isolates from different parts

*Corresponding author. E-mail: zaidi_aijaz@yahoo.com; fax: +911894-230433.

Abbreviations: CaMV = Cauliflower mosaic virus; CarMV = Carnation mottle virus; CERV = Carnation etched ring virus; CP = coat protein; DAS-ELISA = double-antibody sandwich enzyme-linked immunosorbent assay; PCR = polymerase chain reaction; FMV = Figwort mosaic virus; MiMV = Mirabilis mosaic virus; PCSV = Peanut chlorotic streak virus; SVBV = Strawberry vein banding virus

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Table 1. Incidence of CERV disease on carnation cultivars on the basis of symptoms and ELISA

Cv. No.	Carnation cv.	ELISA	Cv. No.	Carnation cv.	ELISA
1	Acca Pola	++	32	Marathona	–
2	Aicardi	+	33	Mercia	–
3	America	++	34	Mona Lisa	–
4	Ariane	–	35	New Espana	+
5	Arthur Sim	+	36	Orange Triumph	+
6	Balade Pink	+++	37	Parado	++
7	Bright Levdevory	++	38	Pink Dona	–
8	Cabaret	++	39	Pintoo	+
9	Charmint	+++	40	Pirandello	++
10	Charmour	–	41	Pleasure	–
11	Cherrio	–	42	Purple Chopin	–
12	Cherry Solar	+++	43	Raggio de Sole	++
13	Dalphe White	–	44	Red Carso	+
14	Dark Tempo	++	45	Rubesco	++
15	Desio	+++	46	Safari	++
16	Dona brecas	–	47	Saleya (X1)	++
17	Dusty Pink	+++	48	Salmanca	–
18	Espana	–	49	Scania	++
19	Firato	+++	50	Shocking pink	+++
20	Flair	++	51	Sonsara	+++
21	Garbo	++	52	Sorriso	–
22	Impala	–	53	Sunrise	–
23	Indios	+	54	Super Green	+
24	Irma	+++	55	Super Star	+++
25	Jack	–	56	Talima	++
26	Jose	+	57	Tasman	–
27	LaSpama	++	58	Trendy	+++
28	Lavender Lace	++	59	White Candy	–
29	Leon	+++	60	White Wedding	–
30	Liberty	++	61	Yellow Candy	+++
31	Madame Collette	+++			

(–) = negative reaction, (+) = weakly positive reaction, (++) = moderately positive reaction, (+++) = strongly positive reaction.

of the world and with other caulimoviruses on the basis of nucleotide and amino acid sequences of selected genome regions. In this way the homology among CERV isolates from two different parts of the world, i.e. Europe and Asia, was determined. Partial nucleotide sequence of the Indian CERV isolate is presented for the first time.

Materials and Methods

Virus isolation. The Indian CERV isolate used in this study was obtained by inoculation of partially purified virus obtained from *Saponaria vaccaria*. Carnation cultivars found negative for CarMV but positive for CERV, were used for partial virus purification described earlier (Covey *et al*., 1998).

Bioassays. *Amaranthus caudatus, Antirrhinum majus, Beta vulgaris, Celosia argentia, Dianthus barbatus, D. caryophyllus, Nicotiana clevelandii,* and *S. vaccaria* were inoculated mechanically in triplicate at four-six-leaf stage. Partially purified viral preparations were used as inoculum. Plants were maintained at 20–25°C, observed for symptoms periodically and further analyzed by DAS ELISA for verification of bioassay results.

ELISA. Sixty-one carnation cultivars grown in Kangra Valley, Himachal Pradesh, India, were screened for the CERV presence by DAS-ELISA (Clark and Adams, 1977) using commercially available virus-specific antibodies (Agdia and Bio-Rad, USA). Five plants, selected randomly from each cv, were analyzed by ELISA. A_{405} was read in a microtitre plate reader (Multiskan EX, Labsystems, USA). The ELISA result was considered positive when the A_{405} of the sample was at least three times higher than the mean A_{405} of healthy controls in triplicate. The A_{405} comparable to the positive control, about half of the positive control and about threefold of the negative control, respectively, was regarded as strongly (+++), moderately (++) and weakly (+) positive (Table 1).

DNA isolation. Total nucleic acid was isolated from 2 g of a leaf tissue using a standard method (Sambrook *et al*., 1989). The pellet was resuspended in 500 µl of nuclease-free water and RNA was removed by LiCl precipitation. Total DNA was ethanol-precipitated, washed, dried and redissolved in 80 µl of nuclease-free water.

PCR amplification. The primers used for amplification were those reported by Sanchez-Navarro (1999). The following antisense

and sense primers were used: 5'-CATCCATTGCTTAgACTTTTC-3' and 5'-CTTTGTGACATTTGCTC-3', respectively. The reaction was carried out in a 50 µl volume containing 150 ng of each primer, 3 µl of 10 mmol/l dNTPs, 5 µl of 10 x PCR buffer (Genei, India), 1.5 U of Taq DNA polymerase (Genei, India) and 200 ng of DNA. The reaction consisted of 39 cycles of 92°C/1 min, 43°C/2 mins, and 72°C/2 mins and a final cycle of 72°C/10 mins carried out in a Mastercycler (Eppendorf, Germany). The PCR products (10 µl) were electrophoresed in 1% agarose gel in 1 x TAE buffer (0.04 mol/l Tris-acetate and 0.001 mol/l EDTA), stained with 0.5 µg/µl ethidium-bromide and observed under UV light.

cDNA cloning, nucleotide sequencing and analysis of the amplicon. The PCR amplified fragment was cloned using the pGEMT-Easy vector system (Promega). The recombinant plasmid DNA for sequencing was isolated by the boiling method (Holmes and Quingley, 1981) and purified by using the WIZARD DNA cleanup system (Promega). Both strands of the cloned DNA were sequenced by the dideoxy chain termination method (Sanger, 1977) using an automated sequencer (ABI PRISM 310, Applied Biosystems, USA) and the ABI PRISM® Big Dye™ Terminator (version 3.0) Ready Reaction Cycle Sequencing Kit (Applied Biosystems, USA). The nucleotide sequences of a portion of genomes of the Indian CERV isolate, another CERV isolate and other caulimoviruses available at the EMBL nucleotide database were analyzed using the BLAST (NCBI) program (Altschul *et al.*, 1997). The BLASTP program was used for searching the amino acid sequence database. The parities comparison was performed using the ALIGN-2 program (Tatusova and Maiden, 1999). Multiple alignments were generated by the MULTALIN program (Corpet, 1988). The nucleotide sequence of the Indian CERV isolate reported in this paper were deposited at the EMBL nucleotide database under the Acc. No. AJ549330.

Results

Bioassays

The partially purified viral concentrate compared to the crude sap enhanced the incidence of CERV infection and thus was routinely used for inoculation. *S. vaccaria*, *D. barbatus* and *D. caryophyllus* proved to be suitable indicator plants under the conditions desribed. Necrotic ringspots or lines developed on *Saponaria vaccaria* while *D. barbatus* and *D. caryophyllus* showed typical "etched ring" symptoms.

DAS-ELISA

DAS-ELISA confirmed the widespread CERV presence in most of the carnation cultivars tested (Table 1). Even the cultivars not showing marked visible symptoms were DAS-ELISA-positive for CERV. Also the movement and extent of CERV infection during the bioassay and host range studies were monitored by DAS-ELISA. The latter proved to be a good diagnostic tool for CERV detection in plants used for maintenance of virus culture and also for cross-checking the presence of other viruses.

PCR amplification and sequence analysis

The DNA fragment of the expected size (1350 bp containing a portion of CP and enzymatic polyprotein of CERV genome, was amplified and sequenced. The fragment contained a 3'-terminal part of the coat protein (CP) gene (nt 1–708) and a 5'-part of the enzymatic polyprotein (nt 705–1349) gene. Nucleotide sequence analysis showed that the part of the CP gene was similar by 97.7% while the part of the enzymatic polyprotein gene was similar by 96% to the corresponding sequences in the database. When the corresponding deduced amino acid sequences were compared, the similarity rose to 98.7% and 97.2%, respectively (Figs. 1 and 2, Tables 2 and 3). When the isolate was compared with other caulimoviruses in the database, it showed a nucleotide similarity of 40–55% in the case of CP and 44–60% in the case of enzymatic polyprotein (Tables 2 and 3).

Table 2. Percent nucleotide (below diagonal) and amino acid (above diagonal) sequence similarities between CPs of different caulimoviruses

	PCSV	BRRV	SCBV	FMV	MiMV	CERV	CaMV	CERV2
PCSV	X	48.4	43.7	41.4	45.9	36.5	42.9	43.2
BRRV	49.9	X	43.4	45.8	41.0	47.3	46.9	51.6
SCBV	45.4	44.4	X	47.8	47.1	49.0	51.7	57.5
FMV	45.7	45.7	49.4	X	56.5	50.8	54.8	57.4
MiMV	41.5	42.0	39.8	50.1	X	50.2	50.9	58.9
CERV1	44.4	46.5	50.6	49.1	44.9	X	62.0	98.7
CaMV	41.6	42.3	45.0	48.3	47.3	47.9	X	71.0
CERV2	49.7	50.4	54.8	52.7	42.4	97.7	40.2	X

For the caulimovirus abbreviations see their list on the front page.
BBRV (Acc. No. NC-003138). CERV1 (from the EMBL database, Acc. No. NC-003498). CERV2 (Indian isolate, Acc. No. AJ-549330). CaMV (Acc. No. NC-001497). FMV (Acc. No. NC-003554). MiMV (Acc. No. 004036). PCSV (Acc. No. NC-001634). SVBV (Acc. No. NC-001725).

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CERV        MSLRNRTNPN SIYVKGILKF PGYQTNLDLH CYVDTGSSLC MASKYVIPEE
CERV2       MSLRNRTNPN SIYVKGILKF PGYK.QLELH CYVDTGSSLC MASKYVIPEE
FMV         H..LNVTNPN SIYIEGKLSF EGYKS.FNIH CYVDTGASLC IASRYIIPEE
MiMV        ...ANKTNPN SIYIEAKVKF KGYQT.LSLH CYVDTGASLC LASKHVIPDD
CaMV        EQVMNVTNPN SIYIKGRLYF KGYKK.IELH CFVDTGASLC IASKFVIPEE
SVBV        FSTLTKTNPN SIYIRGNFYF KGYKK.YSLD LYVDTGASMC TANKHVIPEE
BRRV        ~~~~~~MNQN NTFIKITISQ K......TIL AYIDTGASLC LLPEYNLPKQ
PCSV        ~~~~~MSSKN SSFIKVKLFN K......YLY AYIDTGATIC LAQAKILPIK
Consensus   MSL-N-TNPN SIYIKGKLKF KGYK--L-LH CYVDTGASLC LASKYVIPEE

CERV        YWQTAEKPLN IKIANGKIIQ LTKVCSKLPI RLGGERFLIP TLFQQESGID
CERV2       YWQTAEKPLN IKIANGKIIQ LTKVCSKLPI RLGGERFLIP TLFQQESGID
FMV         LWENSPKDIQ VKIANQELIK ITKVCKNLKV KFAGKSFEIP TVYQQETGID
MiMV        FWENAPRSIP VKIANQSSIT LNKVCRGLKI NIAGNEFFIP TIYQQETGID
CaMV        HWVNAERPIM VKIADGSSIT ISKVCKDIDL IIAGEIFRIP TVYQQESGID
SVBV        FWVNAKNPIR ARIANDSIMT FNKVAELMQV QIADETFIIP TLYQATTKGD
BRRV        LWKELKKPIT IRVADKRELQ INKVALMITI LIEKRKFLVP TIYQFDSGVP
PCSV        YWKKMIKPIK VRIANNKVIH IWYKAVDLVL LLEGKRFPLP SVYQQDAGLP
Consensus   YW-NAEKPIN VKIANG-II- I-KVC--L-I -IAGERFLIP T-YQQESGID

CERV        LLLGNNFCQL YSPFIQYTDR IYFHLNKQS. ...VIIGKIT KAYQYGVKGF
CERV2       LLLGNNFCQL YSPFIQYTDR IYFHLNKQS. ...VIIGKIT KAYQYGVKGF
FMV         FLIGNNFCRL YNPFIQWEDR IAFHLK..NE ..MVLIKKVT KAFSVSNPSF
MiMV        ILLGNNFCQT YQPFIQWVDH IAFHIKENNE EKRVLIPKVR IAMRKGHPGF
CaMV        FIIGNNFCQL YEPFIQFTDR VIF...TKNK SYPVHIAKLT RAVRVGTEGF
SVBV        ITLGNNFCRL YEPFVQYKDM ITFH....KD GRAVSTKKVT KAYFHGLPGF
BRRV        MIIGNNFLRL YYPFCQYLSY ITLRC..... .......... ..........
PCSV        LILGNNFLKL YNPFIQTLET ISLRC..... .......... ..........
Consensus   LLLGNNFCQL Y-PFIQYTDR I-FHL-KQNE ---V-I-KVT KAY--GVPGF

CERV        LESMKKKSKV NRPEPINITS N......QHL FLEEGGNHVD EMLYEIQISK
CERV2       LESMKKKYKV NRPEPINITS N......QHL FLEEGGSNIE ELCYEIQISK
FMV         LENMKKDSKT EQIPGTNISK NIIN...... .........P EERYFLITEK
MiMV        LEAMRKGSGK KPVPGTNITQ EVID...... .........D EEKGFIEISK
CaMV        LESMKKRSKT QQPEPVNIST NKIENPLEEI AILSEGRRLS EEKLFITQQR
SVBV        LESKKVGSST STPNPENITP VTINQDNIS. KIFKGEEIGE SEQLFSTISA
BRRV        .......... ..PKMINQKQ EVIKIPIHHS SQLIKAKLLN LVTNIEEQLL
PCSV        .......... ..PQLEKQPS SLITTKIYNT FSLFGGVIVN ILKQQIYIAI
Consensus   LESMKK-SKT N-PEPINITS NVIN-PIQHL F-LEGG-I-- EEKYFI-ISK

CERV        FSAIEEMLER VSSENPIDPE KSKQWM
CERV2       FSAIEEMLER VSSENPIDPE KSKQWM
FMV         YQKIEQLLDK VCSENPIDPI KSKQWM
MiMV        FREIEQLLEK VCSENPIDPE KSKGWM
CaMV        MQKIEELLEK VCSENPLDPN KTKQWM
SVBV        YTEVEKLLDS ICSEHPLDSR INKGKF
BRRV        MEQVNKILQE RFSLDLLG.E KNKNKE
PCSV        EDEVTQLLEA ICSQNPLDPQ KNRNQI
Consensus   F-EIE-LLEK VCSENP-DPE KSKQWM
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Fig. 1
Multiple alignment of amino acid sequences of the enzymatic polyproteins of different caulimoviruses inclusive of the Indian isolate of CERV
Amino acids in bold show variable positions.

Table 3. Percent nucleotide (below diagonal) and amino acid (above diagonal) sequence similarities between enzymatic polyproteins of different caulimoviruses

	PCSV	BRRV	SCBV	FMV	MiMV	CERV	CaMV	CERV2
PCSV	X	60.2	54.0	54.0	55.4	54.7	55.9	54.6
BRRV	58.3	X	53.1	51.8	51.6	53.1	54.5	60.2
SCBV	51.6	52.7	X	63.4	63.3	65.2	63.2	56.9
FMV	51.5	53.3	58.3	X	78.4	74.6	73.1	66.3
MiMV	51.2	52.5	57.6	67.2	X	73.6	71.8	69.1
CERV	50.5	50.7	58.0	66.0	65.1	X	77.6	97.2
CaMV	50.2	53.2	41.2	64.0	61.9	66.7	X	70.5
CERV2	47.0	43.5	54.0	60.0	58.8	96.1	60.9	X

For the legend see Table 2.

Discussion

CERV is the only DNA virus reported to date in carnation, in which it causes mild symptoms. Yet it is more easily observed in carnation cultivars as a mixed infection with CarMV. In this study, the CERV infection was symptomless at temperatures over 28°C. The symptoms were most visible at 20–25°C, the optimum temperature also for the growth of carnations. Similar observations have also been made earlier by Lawson *et al*. (1977). Our bioassay results were similar to those reported earlier by Hakkaart (1968, 1972) and thus confirmed the CERV presence. The bioassay is an inexpensive but time and labor consuming method which requires considerable knowledge of the virus involved. Thus new target-specific methods may improve the efficiency of control strategies in the future. Therefore rapid, specific and sensitive methods for detection of all-important pathogens are required. On the other hand, for primary screening, maintenance and purification of a virus, we have to rely on the host range and bioassay.

In this study, when our bioassay CERV-positive results were checked by DAS-ELISA, 76% of them were confirmed as positive. These results indicate a wide spread of CERV in carnation cultivars in this part of the world.

Earlier reports on CERV infection in Asia have been based on preliminary observations such as host range, transmission by vectors and maintenance of the virus on tissue culture-raised carnations (Smookler and Loebenstein, 1975; Ram *et al*., 1998). However, there are no such reports on molecular characterization or sequence analysis of CERV isolates from this part of the world. The methods used in this study for virus purification and nucleic acid isolation gave satisfactory results. Although no commercial kit enabling comparison of results was used for DNA isolation, the methods described are cost-effective and easy to perform.

The fact that the sequence similarity of both the partial genes to those available in the database was about 97% suggests that also the Indian isolate has conserved regions in CP as well as enzymatic polyprotein genes. To maximize the sensitivity when comparing coding nucleotide sequences between different CERV isolates or caulimoviruses, translated nucleotide sequences were preferred, since they contained more tightly conserved sequences. The translated nucleotide sequences showed about 98–99% similarity among CP and enzymatic polyprotein genes. Other caulimoviruses were also compared to each other with respect to CP and enzymatic polyprotein genes. They tended to have a 40–50% similarity in the CP gene while a 37–62% similarity in the translated sequences. In the case of the enzymatic polyprotein gene among different caulimoviruses there was a 41–67% nucleotide similarity while the translated sequences showed a 53–79% similarity. One of the interesting features was that CaMV and CERV, despite the 48% nucleotide similarity, had a 62% amino acids similarity in the enzymatic polyprotein.

The deduced amino acid sequence for the Indian CERV isolate was also compared with corresponding translated sequences of other caulimoviruses. For the enzymatic polyprotein many similar short stretches were found between the recent Indian CERV isolate (Acc. No. AJ549330) and another CERV isolate (Acc. No. NC-003498), CaMV (Acc. No. NC-001497), Figwort mosaic virus (FMV, Acc. No. NC-003554) and Mirabilis mosaic virus (MiMV, Acc. No. NC-004036), which indicates that these viruses are closely related. The percentage similarity for this protein was highest with CaMV, lesser with FMV and MiMV and least with Peanut chlorotic streak virus (PCSV).

Similar results were also obtained when the deduced amino acid sequences corresponding to the CP gene were compared although similar stretches were fewer in number.

The translated amino acid sequences of the enzymatic polyprotein gene of the two CERV isolates revealed that they differ at two stretches (aa 24–28 and aa 187–193) and one position (aa 158). There is one missing amino acid at position 25 compared to the data from the database. At other positions, the changed amino acids are either polar (aa 24–

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CERV2       LCDICSLQSF FC**P**YESNLYK LPQNEYPSLV KQYLAKIP.. ...IVGEKAS
CERV        LCDICSLQSF FC**D**YESNLYK LPQNEYPSLV KQYLAKIP.. ... VGEKAS
CaMV        LCDICYLEEF TCDYEKNMYK TELADFPGYI NQYLSKIP.. ...IIGEKAL
FMV         LCNICELDNF TCFYEKQINQ LKFEDFPKWI ELYLGKIP.. ...IIGKQSK
MiMV        LCDICLLDDF TCLFEKNLVH FEMSEMPAWV ETYLRKIP.. ...IVGEISR
SVBV        ICNLCSLESF FCDYETNLLK LPIEEWPKYI EEYIRKIP.. ...FVGMEVL
BRRV        ICDMCEFEEF YCQFIHYYYM LESRERTEYM NVFIQKLPYP LSKTINDEFQ
PCSV        LCDPCYIENF FCQVEANYYK V..TDRTGLL DIVLSKMPPP MVTYIQTQIN
Consensus   LCDICSLESF FCDYEKNLYK L-Q-E-P--- EQYL-KIP-P ---I-GEKA-

CERV2       KRFEEEASAA TSYSLGFAHK LVNEELAKIC ELSKKQKKLK RFNKNCCSTF
CERV        KRFEEEASAA TSYSLGFAHK LVNEELAKIC ELSKKQKKLK RFNKNCCSTF
CaMV        TRFRHEANGT SIYSLGFAAK IVKEELSKIC DLSKKQKKLK KFNKKCCSIG
FMV         ERWDNEKSFT TKYSLAFAKR IIQEEIAKYC DFQRTSKKLK NFSKKCCSKN
MiMV        MIYNETKSPA TTYSLAFATR IVKTEIAKIC EARSKAKQLK RFS.QCCKKL
SVBV        EEYSKQDS.I TKGSLGYAHN LIKAYMEKKC KSLKIKKEIR R..NMCCPKF
BRRV        ..SQKNANLI PDTIRRYSQS NKSIHILLQC TKEQEKMQLI NV.TKCCPKF
PCSV        DPSRTRRILT LGLIRRFAIE YRQ....NLC LRKIEKNYIR NVDPKCCKQL
Consensus   -RF-EEAS-- T-YSLGFAHK -VKEE-AKIC ELSKK-KKLK RFNKKCCSKF

CERV2       E....KPYEY GC....KPSY SKKKKYSKKY KPKYTKYKV. .IR**E**.KKKFS
CERV        E....KPYEY GC....KPSY SKKKKYSKKY KPKYTKYKV. .IR**K**.KKKFS
CaMV        E....ASTEY GC....KKT. STKKYHKKRY KKKYKAYKP. .YKK.KKKFR
FMV         SLDPLVS..F GCRDTKKKDF KKSSKY.KAY KKKKTLKKL. .WKKKKRKFT
MiMV        SINDSENNQF GC...NKPSY ..SSKR.KKY EKSR..RKV. .WKKTKRKFA
SVBV        S...SPETQY GCKPISHKK. AKKQKYKQYY KKKYRLRKPK RWTNSRRKYS
BRRV        EYI...PHKF GCSPNS..SF RRGRKRTKPK YSKYKQRKYH TFKPWYKKKR
PCSV        DDV...PQEY GCS..Q..PY RYKKKR.KP. FRRIKARKY. PYRKWKPKYR
Consensus   E---SKP-EY GCSP-SK-SY SKKKKY-KKY KKKY--RKV- -WKKWKKKF-

CERV2       PGKYF...KP KDKKS..... .EKAKYCPKG KKTCRCWVCN IEGHYANECP
CERV        PGKYF...KP KDKKS..... .EKAKYCPKG KKTCRCWVCN IEGHYANECP
CaMV        SGKYF...KP KEKKG..... .SKQKYCPKG KKDCRCWICN IEGHYANECP
FMV         PGKYFSKKKP EK........ .....FCPQG RKKCRCWICT EEGHYANECP
MiMV        PSKYFKRKSS KKDKN..... .PRKNFCPQG KKKCRCWICS EEGHYANECP
SVBV        GRKLFRRKRD QKEETSQQSP EEKKKFCPQG KTTCRCWICN EIGHFAKDCR
BRRV        .YRMYKRKYQ PKYKQRYWKN KSNQKYCPKG KKDCKCWICQ EDGHYANECP
PCSV        .YKVRRKGYS ........KQ QNKQKTCPRG KKTCRCWICQ EEGHYANECP
Consensus   PGKYF-RKKP KKKKS---K- -EKQKYCPKG KKTCRCWICN EEGHYANECP

CERV2       NRQTS.EKFK LIQIAENYGL EPIENPYEDQ QEICLL.... EQIQLSSSDS
CERV        NRQTS.EKFK LIQIAENYGL EPIENPYEDQ QEICLL.... EQIQLSSSDS
CaMV        NRQSS.EKAH ILQQAEKLGL QPIEEPYEGV QEVFILEYKE EEEETSTEES
FMV         NRKSHQEKVK ILIHGMNEGY YPLEDAYTGN LEVFSMEIIE E....TTSEE
MiMV        NRSKNPERVK ILIKGYQQDY EPVEDMYEGT LHVYSYEY.. D....TDSE~
SVBV        NKSANHNK.. IIEELQSLQL EPVFDLNELK IEEKFWE.LK EVSESSESES
BRRV        NKDKRRDKVK LLEQLSQVNL EPIENDNISE EELWY..... ...LQTDEES
PCSV        NRKINQKKDK YVRMLYSVGY EPIEEDYETD ESLDF..DIY SLTSETDSET
Consensus   NRQ--QEKVK ILQ--EN-GL EPIE-PYEGQ QEV--LEYIE EQI-L-DSES

CERV2       ELDDT**Y**EESS SEESE
CERV        ELDDT**C**EESS SEESE
CaMV        DGSSTSEDSD SD~~~
FMV         ESTTSDSSSS DDEQL
MiMV        ~~~~~~~~~~ ~~~~~
SVBV        EISSD.ESSD SEDLE
BRRV        EEENSSDESE QYFYQ
PCSV        ESENEFEE~~ ~~~~~
Consensus   E---TSEESS SE-SE
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Fig. 2
Multiple alignment of amino acid sequences of the CPs of different caulimoviruses inclusive of the Indian isolate of CERV

Amino acids in bold show variable positions.

28) or bulky hydrophobic or bulky aliphatic (aa 187–193); functional effects of these changes need be investigated.

The amino acid sequence of the CP and enzymatic polyprotein were deduced from the gene sequences and compared to an other CERV isolate and other caulimoviruses. It was found out that within the CERV isolates, the CP sequence was more conserved in comparison to the enzymatic polyprotein. The difference between the two CERV isolates was only at three positions, namely 13, 144 and 256, while between the CERV isolates and other caulimoviruses, enzymatic polyprotein is more conserved.

The data presented here indicate that the CERV isolates separated in time and space present a high genetic stability. Thus our results correspond to those found for other viruses such as Carnation mottle virus (Canizares *et al*., 2001), Citrus tristeza virus (Albiach-Marti *et al*., 2000) and Turnip yellow mosaic virus (Blok *et al*., 1987). High genetic stability of CERV may be due to the facts that CERV isolates have evolved from the same original CERV sequence in carnation cultivars, and because of global market of cut flowers and vegetative propagation they have dispersed around the world.

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