

Molecular Analysis of the 3'-Terminal Region of Lily Latent Carlavirus from *Lilium lancitoium*

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(Received on June 17, 2000)

The 3,000 nucleotides of 3'-terminal region of the genomic RNA of a new isolate of carlavirus from a Korean native lily (*Lilium lancitoium*) was cloned and its nucleotide sequences were determined. The coat protein (CP) gene of the virus showed 72.0% to 72.8% nucleotide sequence identities and 86.9% to 88.0% amino acid sequence identities with those of the four strains (two Korean, one Dutch, and one Japanese isolates) of lily symptomless virus (LSV). Interestingly, different amino acid sequences between the new isolate and LSV strains were located at the N-terminal region of the CP. Pairwise amino acid sequence comparison of the CP gene revealed sequence identities of 22.0% to 71.1% between the virus and other 9 carlavirus species. The 25 kDa and 12 kDa proteins genes of the virus share 30.7% to 76.3% and 31.1% to 85.8% amino acid sequence identities, respectively, with those of 8 other carlaviruses. The 16 kDa protein gene of the virus shares 16.7% to 72.9% amino acid sequence identities with that of 9 other carlaviruses. These data indicate that the virus, designated as lily latent virus (LiLV), is a distinct of the *Carlavirus* genus and distinguished from the known strains of LSV.

Keywords: Lily latent virus, Carlavirus, coat protein, plant virus, lily, *Lilium*.

Lily (*Lilium* sp.) is one of the most popular ornamental plants as a cut-flower in Korea as well as in other countries. It is propagated vegetatively, and virus diseases have caused serious problems for growers. To date, several viruses infecting lilies have been reported in the world (Ahn et al., 1999; Allen, 1972; Beijersbergen et al., 1980; Cohen et al., 1995; Derks, 1995; Kim et al., 1995). The members of the genus are filamentous virus particles, 610-690 nm long and 12-13 nm in diameter, which are transmitted by aphids in a non-persistent manner or by whitefly (Cavileer et al., 1994; Foster et al., 1990). The size of the carlaviral

genomes range from 7.4 to 8.5 kb. They are composed of monopartite, single-stranded, plus sense RNA molecules, and have a poly (A) tract at their 3' terminus and cap structure or a monophosphate at their 5' terminus (Cavileer et al., 1994; Foster et al., 1990; Zavriev et al., 1991). They are encapsidated by a single type of coat protein with Mr of 31,000 to 34,000 Da (Ahn et al., 1999; Cavileer et al., 1994; Foster et al., 1990). The triple gene block (TGB), known to encode for the cell-to-cell movement function in the *Potexvirus* genus (Cruz et al., 1995) occurs also in the carlaviruses genomes (Ahn et al., 1999; Cavileer et al., 1994; Foster et al., 1990; Morozov et al., 1991; Turner et al., 1993; Zavriev et al., 1991). Recently, a virus was isolated from a diseased Korean native lily (*Lilium lancitoium*) showing very mild mosaic and/or no symptoms in Choong Nam Province in Korea, and was identified as a carlavirus (Ryu et al., unpublished data). However, no information exists about the relationship between the virus and other carlaviruses. We have cloned the cDNA for the 3'-terminal region of the new carlavirus, designated as lily latent virus (LiLV) for the molecular characterization of the virus. Here, we report the nucleotide sequence analysis of 3,000 bases of the 3'-terminal regions of the LiLV and its comparison with those of other carlaviruses. Based on the comparisons, the virus is identified as a new species belonging in the carlavirus genus.

Materials and Methods

Virus source, purification of virus and extraction of viral RNA. Korean native lily (*L. lancitoium*) infected with virus was maintained in the light/temperature controlled glasshouse and used for source of virus (Fig. 1). The virus was isolated and purified by extraction and clarification with chloroform, followed by differential centrifugation method (Ahn et al., 1999). Total genomic RNA of the virus was extracted from purified virion particles by the method of Ryu et al. (1995).

cDNA synthesis, cloning and sequence determination. First strand cDNA was synthesized from 10 µg of purified viral genomic RNA using the cDNA synthesis kit (GIBCO BRL) with oligo (dT)-*NotI* adaptor-primer according to the manufacturer's instructions. Double stranded cDNA molecules were synthesized

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Fig. 1. LiLV-infected lily (*Lilium lancitoium*) plant showing symptomless in the leaf part.

and cloned into pSPORT1 vector (GIBCO BRL). Recombinant plasmids were transformed to *E. coli* strain NM522, and recombinant colonies were selected for nucleotide sequencing (Sambrook et al., 1989). Subclones containing the viral genes were generated by restriction digestion. Overlapping clones were generated by the unidirectional deletion procedure using exonuclease and S1 nuclease (Promega). Nucleotide sequence of each clone was determined in both directions by the dideoxynucleotide chain termination method (Sanger et al., 1977) using the Model 377 automatic DNA sequencer (ABI) and/or by manual sequencing with silver staining (Promega). Nucleotide sequences and deduced amino acid sequences of the virus genomic RNA were analyzed using the DNASTAR software package (Madison, WI).

SDS-PAGE and western blot analysis. Antibody against LiLV was produced from the New Zealand white rabbit by using the purified virion particles and Freund's adjuvant. Purified virion particles were separated on 12% SDS-PAGE (Ahn et al., 1999), and transferred onto nitrocellulose membrane and probed with LiLV CP antibody for western blot analysis (Sambrook et al., 1989).

Results and Discussion

Biological properties, virus purification and morphology of the virus particles. Virus, denoted as Lily latent virus (LiLV), was isolated from diseased Korean lily plant (*L. lancitoium*) (Fig. 1) by combination methods of polyethylene glycol precipitation, differential centrifugation and sucrose density-gradient centrifugation (Ahn et al., 1999). Purified virus particles, as observed by the electron microscope, were filamentous and 680 nm in length and 12 nm in

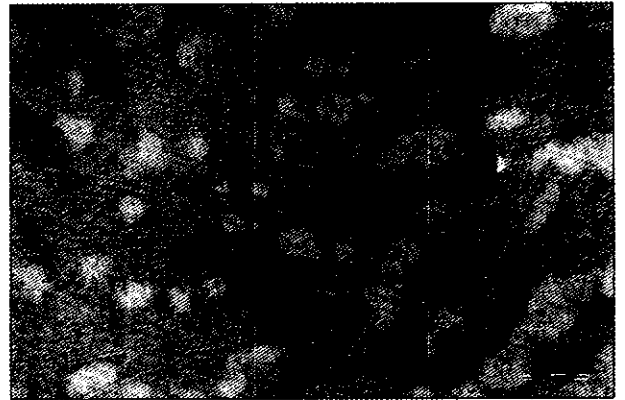


Fig. 2. Electron micrograph of purified virus particles of the lily latent virus (LiLV) stained with sodium phosphotungstic acid. The scale bar represents 300 nm.

width, which is typical morphology of the members of the genus *Carlavirus* (Fig. 2). The virus could systemically infect to *Lilium* plants, but failed to infect in other tested plants (*Datura stramonium*, *Vigna unguiculata*, *Nicotiana tabacum*, *N. benthamiana*, *N. glutinosa*, *Chenopodium quinoa* and *C. amaranticolor*). This demonstrates that isolated LiLV has very narrow host ranges.

SDS-PAGE and western blot analysis and RNA isolation. Purified virions were analyzed by SDS-PAGE and western blotting methods. SDS-PAGE analysis of the viral coat protein (CP) showed a predominant single band of Mr 32 kDa (Fig. 3). In western blot analysis, the protein was detected with antibody raised against purified virions (Fig. 3), indicating that the 32 kDa protein is the viral CP. Genomic RNA of the virus was obtained from purified virion and analysed. When purified viral RNA preparation was separated in agarose gel under denaturing conditions, a single species of 8.0 kb single-stranded RNA molecule was

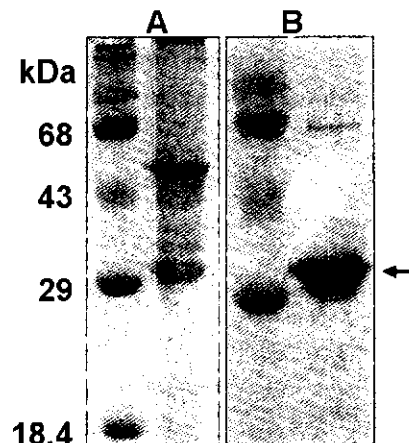


Fig. 3. SDS-PAGE and western blot analysis of LiLV coat protein. Coomassie stained gel (A) and immuno-probed NC membrane with virus-specific antibody (B).

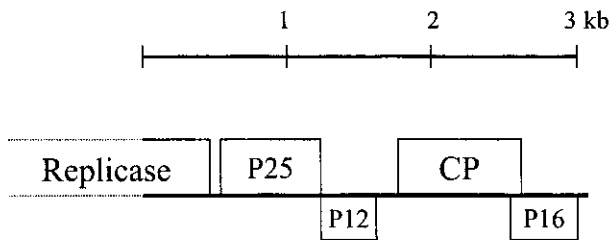


Fig. 4. Diagrammatic representation of the genome organization in the 3'-terminal region of LiLV RNA.

observed (data not shown). The sizes of the CP and RNA are in the range of members of the *Carlavirus* genus (Ahn et al., 1999; Cavileer et al., 1994; Foster et al., 1990; Zavriev et al., 1991).

cDNA cloning of the 3'-terminal region of the virus and sequence determination. The recombinant clone pL005 containing 3.0 kb of the viral genomic cDNA was used for nucleotide sequence determination. The nucleotide sequences were determined from at least two independent clones that were sequenced from both strands. The pL005 covered the 3'-terminal region of the virus and contained a part of the viral replicase gene and full-lengths of four putative open reading frames (ORF) encoding P25 (25 kDa), P12 (12 kDa), and P32 (32 kDa) and P16 (16 kDa) proteins for the

virus (Figs. 4 and 5). The nucleotide sequence reported in this paper will appear in the GenBank/EMBL/DDBJ Nucleotide Sequence Databases under the accession number AJ131812.

Sequence comparison of the viral genes. The nucleotide sequence analysis revealed that the 32 kDa protein gene is the viral CP gene for the LiLV, and this correlate with the results of SDS-PAGE and western blot analysis in this study. The CP cistron for LiLV consisted of 873 nucleotides which encodes 291 amino acid residues with a 31,905 Da. The CP gene of LiLV showed 72.0% to 72.8% nucleotide sequence identities and 86.9% to 88.0% amino acid sequence identities with those of the four strains (two Korean, one Japanese and one Dutch isolates) of lily symptomless virus (LSV) (Ahn et al., 1999; Memelink et al., 1990; Takamatsu et al., 1994). Interestingly, different amino acid sequences between the virus and LSV strains were located at the N-terminal region of CP gene (data not shown). Pairwise amino acid sequence of the CP comparison revealed sequence identities of 22.0% to 71.1% between the virus and other 9 carlavirus species (Table 1). The phylogenetic tree analysis by CP comparison indicates LiLV is closely related with LSV, blueberry scorch virus (BSV) and potato virus S (PVS) (Fig. 6).

The 25 kDa and 12 kDa proteins genes of the virus shares

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1  AAAGTCGGTAACCTTGCACATATGCGTTTCTCAGGTGAAGCGAGCACTTTTCTATTCAAC
   K L G N F A I M R F S G E A S T F L F N
61  ACTATGGCCACATGCTCTTCTACCTTTTGGAGTATGAGATCAAGGGCGATGAACGGGATT
   T M A N M L F T F L R Y E I K G D E R I
121  TGCCTCGTGGAGATGATGTGTTTAAACAAAATTTATCATCTCCGAGAGCAAGCT
   C F A G D D M G S N K R L E T I S Q K H A
181  GATTTCTGGCAGCTCAAAATGAAAGCAAAAGTGCCTACACTAAAGCCCAAGATT
   D F L G K L K K A K V A H T K T P T F
241  TCCGTTGGAATTTGCCCCACGGGATTTAAGAAACCGCAATGGTGTGGAGAGA
   C G W N L C P H G I F K K P Q L V L E R
301  TTGTGATGCCCAAGGAGCAATAAATGCTGCACTGACACTATGCCATGAA
   L C I A K E T N L V N C I D N Y A I E
361  GTCCGATTTCTTCAAGATGGGAGAGAGCGAGAGAGAGAAATGAATGAGGAGAACTC
   V A F A V K M G E K A R E R M N E E L
421  GACCGATTTTACACTCGTTCGATCATAGTTAAATAAAGCACTCTTGAAGTCGGAT
   D A F Y N C V R T I V K N K H L L K S D
481  GTCGAGAGGTGTATGAGGAGCGAGCTGAAGGTAGCTTAGTATTACCAATAAATATG
   V R E V V E E R A E R *
541  TATAATGATGCTTACTAAGTTTGTGAGTATTGTTTGGAGGTTGAGTAGCGAG
   25 kDa M D V L F D I G L V D A E P Q G T V L
601  TTAACCTACCAATGTAATACATAGTTCGCCGGCGGGGTAATCAAGTTTAATAGG
   L T L P P I V I H S V P G G G K S S L I R
661  GCTTTAATAAACAAGATAGCGTTTTCAGCGTTTCACATTCGCGAAGGAGATGCGAG
   A L I N K F D K R F A A F T F G K E D C E
721  AATATACAGAGTCAAGTCAAGAGAGTCCCGAGGTGTTGCAACAATGAAATTTGCA
   N I T G V R I K K V P E V L Q Q I E F A
781  CTCTTTGAGAGTACTTGAAGGGCGTGGCGACTGGGTTTGTCTGTGTCTGAC
   L F D E V L E G A F V P T W A F A F A D
841  CCATCCAAAGGGGACCGGCACTGTCRGGCGCACACTTATCAAGCGCAAGTCCCAT
   P L O G G P G T V R R A H F I K R K S H
901  CCGTTCGCAAGTCACTGCCAGCTCTTGAAGTACGCTGGTGTGAGGCGAG
   R F G K A C T A Q L L T E L G Y A V E A E
961  GGGACGAGCTCTCAAAATTCAGGGCTTTAGCGCTGAGGCTCAAGTACTGTCTA
   G N D V L F D I G L V D A E P Q G T V L
1021  TTTTACCAAGCAAGTGTGGTACTTACCGAGCACTCCGTCGCTACTGTATT
   F Y E F E V G E L L R O H S V P A V C I
1081  AGCGAGATTCGAGCAATCTTCAACAGGCTACTTCTGCTACTGGTGAATCCTACT
   S E I R G G S F T S V T F V T G E S Y P
1141  GTCCAGCGGCTTACCTTCCAGTCCGCTGACCGCCACTTCTCATCTCTTAATACIT
   V D R A L A F Q C L T R H C S L L I L
1201  TGCCCAATGCCACTTACACCCTGATTACACACCGGTSTACCTAGCATTGCCAT
   C P N A T Y T T A *
12 kDa
1261  W P L T P P D V T R V V V A F A I
   CGAGCACTCACTTCAAGTGTGGTACACAGCACTTACCCACTGTGG
   G A S T A L E F T G L V T R S T I P T V G
1321  CGACTTGCAGCAACTCCGACGAGGTGCTACCGGAGGCTACTAAAGCGTGA
   D L Q H N L P H G G R Y R D G T K S V E
1381  GTACTGCGGCGGAAAGACTCAACTACGTGGAAAGCACTGGCGTGTGGCAAAAGCC
   Y C G P R K L N Y V E S T G R W H N Q P
1441  CTGGCTTCTAGTATGACTGCTGCGCACTAATCATAGCCATGGGCGCCAAAGCCAC
   W L L V I V L V A L I I A I G R G S H T
1501  TTCCAGAGTGTGCGTCAATGCTGATGTTGTTGACACAGCATCTCGGGGTATATGTT
   C R V C G R S H *
1561  AGTTACAGCTTCTGAAATACCAAAATCGAAACCTGACCCCTTACATCACTGGCCAA
    
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1621  TCGATACGGATTACTGGTTGCAACTTGAGCCAGCTCACCTACGCGCAGTGCACACCTA
1681  AAAGTGCTACAAGTGGCGCTTAGTAAACAGGCTGGAAGGCGATAAAACAACACTTGTAA
1741  TTCAACATGCAATCAGCTCAAGCCAGATTCGGGCGCTGCAGCAGAAACTTCAGATCG
   32 kDa H Q S Q S K P D S G A A A E T S D P
1801  ACTCGAGTGGCCAGCGGTCCAAGTCTGACGACAGAGTGCAGTGAATAAATCAAGCG
   T R G G R A G G P S P D E R G S E N N A
1861  GAGTCCATACTGAGCAGCGTTTACTAGGCTAATCGAAGCTGAAATGAAAAAC
   E S I L E Q R L L T R L I E R L N A E K H
1921  AACTCCAATCTCGCAATGGCTTTCGAGATCGGTAGACCTAGCTTGGAGCCCAAGC
   N S N L R N V A F E I G R P S L E P T S
1981  ACAATGCGACGCAACCCGCAATCTTACGGGCGATTTCATCGATGAGCTGTTTAAA
   T M R R N P A N P Y G R F S I D E L F K
2041  ATGAAGTGGGGCTGCTGCAATAACATGGCGCACTGAAACAAATGGCAAGATAGCA
   M K V G V V Y S N N H A T G T E Q N A K I A
2101  TCAGACATCGCGGTTTGGAGTCTCTCGAGCAGTCTGATCTGATTAATCTCAGATG
   S D I A G L G V P T E H V A S V I I Q M
2161  GTCATCATGTGCGCATGCTGAGCAGCTCTGATTTCTTACCGGAGTGGTGCATCGAG
   V I M C A C V S S S V F L D D P D G S I E
2221  TTTGATAACGGTGTGTCGCCGTTGACCTCGATGACGCTATAATGAAGAAACATGCCGG
   F D N G A V P V D S I A A I M K H A G
2281  CTGGCAAGTGTGCGGTTTGTATGCAACCTGTTTGGAAACAGCATCTCTGTAAGGAT
   L R K V C R L Y A P I V W S M L V R N
2341  CAACCAAGCGGATGGCGAGGCTTCCATACACACAGGATTCGCGGCTTC
   G P P A D W Q A H G F Q Y N T R F A F
2401  GATACCTTCACTACTTACCAAGCGCGCTTCCAGCGAGTGGAGGAAATCAITCGC
   D T F D Y V T N O A A I O P G E I R
2461  CGAACCACATCTGCGAGGCTATTGCGCATAACGACACAAACAACTCGCGCTCAGCAGA
   R P T S A E V I A H N A H K Q L A L D R
2521  TCTAATCGCAATGAGCGCTCGGGAACTGAGGCGGATTACACTGGTGAATTCAGGT
   S N R N E R F G N L E A D Y T G G I Q G
   16 kDa
2581  GCAGAGATCGTCCGAAACCCGATTTGCAAAACATGGATTAAGCAATAAAGCGAGTGT
   A E L V R N H R F A N G *
   Q R S S E T T D L Q T M D E A N K R R V
2641  GACAATTGCTGTGTTACTTAAGTCTTCCGCGGTGATGTGGCAATATATTAAG
   T I V L C L L K V F P R D V C N I I L R
2701  GCAAACCTTAGCCAGTGTGTTGGTTTCCGCAAGTCAAGATGCTGCGACGCTCGCGGC
   Q T S S H V V G F G K S K Y A R R R R A
2761  CCAGCAATGGCAGGTGTGAGAGGTGTACCGAATATTCACACCGCTTTCGGGTTCTAA
   G I G R C E R C Y R I K
2821  GTGTGACAAATAGACTGCAAGCGGTATCTCTATAAATACAAATGGCCAACTTCAT
   C D H K T C R P G T S I N T N V A N F I
2881  TGATCATGGCGTAACTGAGGCGATGATCAAGCCCAACCGGAAACGTTACTCT
   D H G V T E V R P W I T P N R N K F Y L
2941  AAGACTTAAATAATAGCGTAAACTATAAAATAAATTTGTTTAAATATTTTGA
   R L K *
    
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Fig. 5. Nucleotide and amino acid sequences of the 3'-terminal region of LiLV RNA. The 32 kDa protein is the LiLV CP gene.

Table 1. Percentage sequence similarities of deduced amino acid sequence of P25, P12, P32 (CP) and P16 of LiLV with respective proteins of other carlaviruses^a

| Virus | P25 | P12 | P32 | P16 |
|---------|----------------|------|------|------|
| LSV-Ne | 76.3 | 85.8 | 88.0 | 72.9 |
| BISV | 59.6 | 64.2 | 71.1 | 41.4 |
| CLV | — ^b | — | 37.5 | 16.7 |
| CVB | 46.9 | 52.8 | 44.3 | 37.4 |
| GLV | 32.0 | 38.7 | 34.7 | 32.7 |
| HVS | — | — | 42.6 | 42.7 |
| PopMV | 30.7 | 41.5 | 30.9 | 25.6 |
| PVM | 47.4 | 50.0 | 46.0 | 38.9 |
| PVS | 53.3 | 62.3 | 65.6 | 43.0 |
| RSPaV-1 | 31.7 | 31.1 | 22.0 | 35.7 |

^aReferences for sequences are as follows; LiLV (in this study); PVS (Mackenzie et al., 1989); PVM (Zavriev et al., 1991); CLV (Meehan and Mills, 1991); CVB (Levay and Zavriev, 1991); BISV (Cavileer et al., 1994); GLV (Tsuneyoshi and Sumi, 1996); HVS (Foster et al., 1990); LSV-Ne (Memelink et al., 1990); PopMV (Henderson et al., 1992); RSPaV-1 (Meng et al., 1998).

^b—: data not available for analysis.

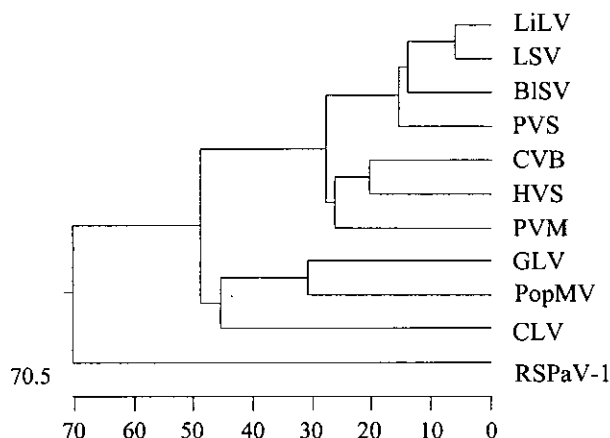


Fig. 6. Phylogenetic tree analysis of complete amino acid sequences of the coat protein gene of LiLV with the corresponding proteins of 10 members of carlaviruses. ^aReferences and full names of each virus for sequences are as follows; LiLV (in this study); PVS (Mackenzie et al., 1989); PVM (Zavriev et al., 1991); CLV (Meehan and Mills, 1991); CVB (Levay and Zavriev, 1991); BISV (Cavileer et al., 1994); GLV (Tsuneyoshi and Sumi, 1996); HVS (Foster et al., 1990); LSV-Ne (Memelink et al., 1990); PopMV (Henderson et al., 1992); RSPaV-1 (Meng et al., 1998).

30.7% to 76.3% and 31.1% to 85.8% amino acid sequence identities, respectively, with those of 8 carlaviruses (Table 1). The 16 kDa protein gene locating 3'-proximal part of the virus shares 16.7% to 72.9% amino acid sequence identities with those of 9 other carlaviruses (Table 1). The zinc-finger nucleic acid-binding domain, four Cys residues of C-X₂-C-X₁₂-C-X₄-C (Berg, 1986), was found in the 16 kDa protein of LiLV in a pattern of C-X₂-C-X₁₁-C-X₄-C (Fig. 7).

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LiLV  C E R C Y R I F P P V C - - G S K C D N K T C
LSV   C E R C Y R V Y P P V C - - G S K C D N K T C
BISV  C E R C Y R V F P I - - - G A S K C Y N R T C
CLV   C P R C A R V S P G F Y - F T T R C D G K T C
CVB   C H R C Y R V Y P P L F P E I S R C D N R T C
GLV   C P R C F R C S P G F Y - F T K N C D T K S C
HVS   C E R C Y R V Y P P L - P F S K K C D N R T C
PopMV C V R C F R V N P G F Y - F T K R C D G I T C
PVM   C H R C Y R L W P P T V - F T T R C D N K H C
PVS   C W R C Y R V Y P P V C - - N S K C D N R T C

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Motif C X X C X X X X X X X X X X X X X X C X X X X C

Fig. 7. Alignment of zinc-finger nucleic acid-binding domain, four Cys residues of C-X₂-C-X₁₂-C-X₄-C, between LiLV and other carlaviruses.

The 16 kDa protein of carlaviruses may play a role in the transcriptional regulation of host gene due to its nucleic acid-binding property, but the exact role and other possible functions of the protein in the infected cells still remain unknown. In conclusion, our data indicate that the virus, designated as LiLV, is a distinct *Carlavirus* member.

Acknowledgements

This study was supported in part by a Research Grant of National Research Laboratory of KISTEP, in part by Plant Virus GenBank Project (Special Research Inframaterial Program) from the Korea Science & Engineering Foundation in Korea, and in part by 2000 year grant from Natural Science Institute of Seoul Women's University.

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