Journal of Proteomics & Bioinformatics - Open Access

Research Article
OPEN ACCESS Freely available online
doi:10.4172/jpb.1000141
JPB/Vol.3 Issue 6

Inference on Coat Protein Evolution of *Lily Symptomless Carlavirus* in India and Abroad Based on Motifs Study and Phylogenetic Analysis

Suhasini Huddone[1#*], Satya Vrat Bhardwaj[1], Rameshwar Singh Rattan[2##], Kamlesh Kanwar[1], Gaurav Zinta[1###] and Anil Handa[3]

[1]*Department of Biotechnology, Dr Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan (Himachal Pradesh) 173230, India*
[2]*Department of Entomology, Dr Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan (Himachal Pradesh) 173230, India*
[3]*Department of Plant Pathology, Dr Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan (Himachal Pradesh) 173230, India*
[#]*Current address: Department of Molecular Biology, Central Potato Research Institute, Shimla (Himachal Pradesh) 171001, India*
[##]*Current address: Institute of Himalayan Bioresource Technology, HATS, Palampur, (Himachal Pradesh) 176061, India*
[###]*Current Address:- Department of Biology, University of Antwerp, Universiteitsplein 1, B 2610 Wilrijk, Belgium*

Abstract

Lily symptomless carlavirus (LSV), the most common lily infecting virus around the world, contains 6 open reading frames (ORFs) in its genome, of which ORF5 representing coat protein (CP) is the most variable region and is used here to deduce phylogeny of the virus. CP gene of one of the LSV isolates present in the region, LSV isolate-Oh (Accession no. AJ748277) was taken as test sequence.Multiple sequence alignment of test sequence with ClustalW showed nucleotide and amino acid homology of up to 17-98% and 1-98%, respectively with other 78 carlaviral sequences from India and abroad. One conserved nucleotide motif of carlaviruses, AATAAA (Polyadenylation signal motif) was searched for, in the multiple sequence alignments but it was not found in any of the LSV isolates under study. Further, phylogenetic analysis of nucleotide sequences by DNADIST method of Neighbor-joining algorithm placed test LSV isolate most closely to its native LSV isolates from India and, LSV isolates Yunnan and Lanzou, from China. It could be interpreted that in *Lily symptomless carlavirus* at nucleotide level, evolution is taking place at a faster pace. Also, this virus shared its most recent common ancestry (MRCA), both with its native LSV isolates from India and as well as with LSV isolates from China, probably, indicating its origin from either of the countries. This study provides important clues about spread of the virus and to the best of our knowledge it is the first detailed study of LSV coat protein gene performed at nucleotide level.

Keywords: LSV; Coat protein; Phylogenetic analysis; Multiple sequence alignment; Coat protein motif; Neighbor-joining

Introduction

Lilium crop has been reported to be susceptible to around twenty viruses under natural and glasshouse conditions (Lee, 1992). The three viruses, associated with most lily viral diseases are aphid transmissible *Lily symptomless* (LSV), *Tulip breaking* (TBV) and *Cucumber mosaic* (CMV) carlaviruses (Allen, 1975), but the most common virus diseases in *Lilium* are caused by LSV alone or in combination with mixed infections of *Cucumber mosaic cucumovirus, Lily mottle potyvirus* and *Tulip breaking potyvirus* (Allen 1972; Brunt et al., 2000; Derks and Asjes, 1975; Derks, 1995). LSV is a member of *Carlavirus* genus which includes more than fifty viruses. LSV infection results in unmarketable flowers and severe reduction in bulb size leading to a drastic reduction in economic returns (Asjes, 2000). LSV is aphid transmissible virus (Brierley and Smith, 1944a; Brierley and Smith, 1944b; Brierley and Smith, 1945) infecting lilies naturally. Various lilies, namely *Lilium longiflorum, Lilium tigrinum*, Asiatic hybrid lily, Oriental hybrid lily, etc. grown in Himachal Pradesh, India, have been found to exhibit various viral symptoms like yellowing, chlorotic striping, vein clearing and deformed flowers. So, it will be significant to know about the evolution of this virus in a way which could lead us to stop it from spreading.

Carlaviruses are the large genus of plant viruses. The genome is a single stranded RNA 7.4-8.5 Kb in size (Cavileer et al., 1994; Fugi et al., 2002; Zavriev et al., 1991) and comprises six ORFs, encoding, in order, the replication related proteins, the putative movement proteins (MP) i.e. triple gene block (TGB), the coat protein (CP) and a putative nucleic acid binding regulatory protein (NABP). CP subunits are of one type, and 31-36 KDa in size (Adams et al., 2004). The carlaviral genomes have a poly (A) tract at their 3´- terminus and a cap structure or a monophosphate at their 5´- terminus (Zavriev et al., 1991). The genus comprises of more than 50 viruses such as *Chrysanthemum virus* B (CVB), *Potato virus* M (PVM), *Hop latent virus* (HpLV), *Lily symptomless virus* (LSV), *Daphne virus* S (DVS), *Helenium virus* S (HelVS), *Garlic common latent virus* (GarCLV), etc. Among these viruses, LSV is found to be a major threat for the *Lilium* industry.

LSV was first reported in *Lilium* species from Oregon, USA by Brierley and Smith in 1944. Today around the world, LSV has been reported from different countries of USA, Europe, Asia and Australia (Asjes, 1998). LSV is transmitted by *Myzus persisae, Macrosiphum euphorbiae, Aulocorthium solani, Aphis gossypii, Aphis fabae* or by whiteflies also. This aphid-borne carlavirus is unique to its genus as the plants infected with this virus show no symptoms at its initial stages of development, which leads to a problem in early detection of this virus. We describe here the comparative analysis of this particular virus because it is essential to prepare knowledge based design strategies for controlling these types of viruses.

This study conducted in light of CP gene sequences of various

***Corresponding author:** Suhasini Huddone, Department of Biotechnology, Dr Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan (Himachal Pradesh) 173230, India, E-mail: suhasinihuddone2010@gmail.com

Received June 11, 2010; **Accepted** June 29, 2010; **Published** June 29, 2010

Citation: Suhasini H, Bhardwaj SV, Rattan RS, Kanwar K, Zinta G, et al. (2010) Inference on Coat Protein Evolution of Lily Symptomless Carlavirus in India and Abroad Based on Motifs Study and Phylogenetic Analysis. J Proteomics Bioinform 3: 204-211. doi:10.4172/jpb.1000141

carlaviruses present throughout the world, which is a function of viral assembly and behavior, is the first to show a detailed analysis of coat protein of this important species of plant viruses. Comparisons of the CP gene of carlaviruses have led us to hypothesize about the probable importance of China or India as a source of diversity and evolutionary change with respect to LSV. The investigations also indicated high level of variations in the test LSV isolate (Accession no. AJ748277) occurring at nucleotide level as compared to amino acid level.

Methods

Sequence selection for comparative analysis of coat protein gene of lily symptomless carlavirus with other carlaviruses

NCBI (National Centre for Biotechnology Information) database (http://www.ncbi.nlm.nih.gov/) was searched for all the carlaviral CP gene sequences (nucleotide sequences) present (Table 1). One of the nucleotide sequences of coat protein gene from a regional LSV isolate was selected from NCBI database as the test sequence, as it shared 100% homology with one of the LSV isolates sequenced in our lab. This sequence of LSV isolate LSV-Oh (Accession no. AJ748277) selected, was 882 base pair long (Singh et al., 2005). All these sequences were available in GenBank format in NCBI so these were converted into 'Fasta' (Pearson, 2000) format for further experimentations.

Multiple sequence alignment and motifs search

Comparative studies of nucleotide sequence of coat protein gene of LSV (Accession no. AJ748277) with that of other carlaviruses were carried out separately for each country except for Brazil and USA. As there was only one CP gene nucleotide sequence available from Brazil in NCBI database, so it had to be grouped with the two CP gene sequences from USA, so that a multiple sequence alignment could be generated. ClustalW program available at Network Protein Sequence Analysis (NPS@) web server, PBIL (Pole Bio-Informatique Lyonnais), Lyon; France (http://pbil.univ-lyon1.fr/) was used to see various conserved regions among the nucleotide and amino acid sequences of CP gene of carlaviruses from different countries and a data of Percentage similarity score for alignments of different countries was also obtained using EBI Tool: ClustalW (http://www.ebi.ac.uk/Tools/clustalw/index.html). Thus, six multiple sequence alignments for CP nucleotide sequences were generated, each for Brazil and USA; Canada; China; India; Japan and South Korea and another six were generated for the amino acid sequences. One already known nucleotide motif, AATAAA (Polyadenylation signal motif), was searched manually in the multiple sequence alignments of carlaviral CP nucleotide sequences, as there was no software available to us which contain the database for motifs of carlaviruses.

Phylogenetic analysis of ORF5

EXOME-HORIZONTM software (Mascon Global Ltd., New Delhi) was used to construct Maximum Likelihood (ML) trees (using PHYLIP package and Dnaml program) for CP nucleotide sequences from different countries, separately. Further, Neighbor-joining (NJ) method of EBI Tool: ClustalW was used to construct a single combined phylogenetic tree for all the carlaviral CP nucleotide and amino acid sequences, seperately. Neighbor-joining method was employed because EXOME-HORIZON™ available to us did not accept large amount of sequence entries so, NJ of ClustalW was the freely available and reliable option.

Results and Discussion

A total of 79 complete coding carlaviral CP sequences were selected from NCBI database and one of the sequences (LSV isolate-Oh, Accession no. AJ748277) was selected as the test sequence and used in our studies. The multiple sequence alignment of CP gene nucleotide sequence of Lily symptomless virus isolate LSV-Oh (Accession no. AJ748277) with that of other carlaviruses from different countries using ClustalW program available at NPS@ web depicted that carlaviruses from Canada showed maximum identity of their residues (47.77%) with the test sequence, while least being shown by sequences from Brazil and USA (10.25%) (Table 2).

Individually, percentage similarities score of the test sequence with the other carlaviruses from around the world was identified using EBI Tool: ClustalW and the test sequence shared maximum similarity of 38% with *Cowpea mild mottle virus* CPMMV-M Accession no. AF024629) from USA; 38% with PVM isolate CL3 (EF063384) and PVM isolate Ca 508 (EF063388) from Canada; 98% with LSV isolate Lanzhou (DQ531052) and 97% with LSV isolate Yunnan (AY326460) from China; 98% with LSV isolate LSV-LL (AJ748320), LSV-A (AJ831415 and AJ831416) and LSV-A3 (AJ831417) and 97% with LSV isolate Palampur (AJ585052), LSV-SL (AJ780923), LSV-Lt (AJ781318), LSV isolate Palampur (AM087400) and LSV isolate Tulip (AM087401 and AM087402) from Palampur, India ; 32% with *Garlic latent virus* (GLV) clone GI-3C2 (AB004686) and *Shallot latent virus* (SLV) clone SI-4C2 (AB004802) from Japan and 44% with three DVS isolates: Kr9 (AJ971469), Kr10 (AJ971470) and Kr11 (AJ971471) from South Korea.

The predicted amino acid sequence of protein products of the CP genes of all the 79 carlaviruses were deduced from nucleotide sequences using 'ExPASy' translate tool (http://us.expasy.org/tools/dna.html). The multiple alignment of CP amino acid sequences performed separately for different countries using ClustalW program of NPS@ web server again depicted that sequences from Brazil and USA shared minimum identity of amino acid residues (1.06%) and those from Canada showed maximum identity of their residues (43.75%) with the test sequence (Table 3). EBI Tool: ClustalW showed widest range of percentage similarities i.e. 1% to 98% between translated amino acid sequences of test CP of LSV and rest of the carlaviral CP sequences from around the world.

The genome organization of HpLV was found to be similar to that of *Potato virus* M (PVM), rather than to sequences of other carlaviruses in earlier studies (Hataya et al., 2000), and similar results were depicted in our results where test LSV isolate exhibit less similarities (41-42% and 45%) at nucleotide and amino acid levels, respectively, with that of 7 *Hop latent virus* isolates from China (Xinjiang province), and only 37-38% and 46-47% of identities with PVM isolates at nucleotide and amino acid levels, respectively. It may be concluded that HpLV and PVM sequences are more identical. CP gene of *Daphne virus* S (DVS) shared 45.5% and 49.5% identities with LSV at amino acid and nucleotide levels, respectively in a report published earlier (Lee et al., 2003). In our investigations also we obtained almost similar findings, where 10 CP sequences of DVS isolates from South Korea, under study, were 39-44% and 46% identical to the test CP sequence of LSV isolate at nucleotide and amino acid levels, respectively. The results were in line with those obtained earlier (Singh et al., 2005), which revealed that LSV-T (LSV isolate obtained from *Lilium tigrinum*; AJ781318) had sequence homology value of 78-84% with the Indian isolates and it shows maximum relatedness of 85% with LSV-C (AJ564640) from China when compared to LSV isolates characterized from other regions of the world.

The present studies also demonstrate that almost all the CVB isolates shared similar homologies i.e. 34-46% and 37-45% with the

Research Article
OPEN ACCESS Freely available online
doi:10.4172/jpb.1000141
JPB/Vol.3 Issue 6

Country	Place	Accession No.	Description (Coat Protein gene)
Brazil		AF228416	*Garlic common latent virus*
Canada		EF063383	*Potato virus* M isolate CL1
		EF063384	*Potato virus* M isolate CL3
		EF063385	*Potato virus* M isolate CL 4
		EF063386	*Potato virus* M isolate Ca 5
		EF063387	*Potato virus* M isolate Ca 128
		EF063388	*Potato virus* M isolate Ca 508
		EF063389	*Potato virus* M isolate Ca 513
China		AF314147	*Garlic latent virus*
		AY326460	*Lily symptomless virus* isolate Yunnan
		DQ531052	*Lily symptomless virus* isolate Lanzhou
	Xinjiang	EF202598	*Hop latent virus* isolate CH1
	Xinjiang	EF202599	*Hop latent virus* isolate SW8
	Xinjiang	EF202600	*Hop latent virus* isolate TY4
	Xinjiang	EF394781	*Hop latent virus* isolate K6
	Xinjiang	EF394782	*Hop latent virus* isolate YLA
	Xinjiang	EF394783	*Hop latent virus* isolate TE2
	Xinjiang	EF394784	*Hop latent virus* isolate AW3
India	Palampur (H. P.)	AJ564858	*Chrysanthemum virus* B
	Jharkhand	AJ580930	*Chrysanthemum virus* B
	Patna	AJ580931	*Chrysanthemum virus* B
	Hyderabad	AJ580954	*Chrysanthemum virus* B
	Palampur	AJ580955	*Chrysanthemum virus* B isolate Luknow
	Ludhiana	AJ580956	*Chrysanthemum virus* B
	Maharashtra	AJ581993	*Chrysanthemum virus* B
	Palampur	AJ585051	*Chrysanthemum virus* B isolate Tamil Nadu
	Palampur	AJ585052	*Lily symptomless virus* isolate Palampur
	Bangalore	AJ585240	*Chrysanthemum virus* B
	Palampur	AJ585514	*Chrysanthemum virus* B
	Delhi	AJ619742	*Chrysanthemum virus* B
	Jaipur	AJ619743	*Chrysanthemum virus* B
	Calcutta	AJ619744	*Chrysanthemum virus* B
	Chandigarh	AJ621814	*Chrysanthemum virus* B
	Siligudi (W. B)	AJ621815	*Chrysanthemum virus* B
	Haryana	AJ629843	*Chrysanthemum virus* B
	Palampur	**AJ748277**	***Lily symptomless virus* isolate LSV-Oh**
	Palampur	AJ748320	*Lily symptomless virus* isolate LSV-LL
	Arunachal Pradesh	AJ748852	*Chrysanthemum virus* B
	Gwaliar	AJ748853	*Chrysanthemum virus* B
	Palampur	AJ780923	*Lily symptomless virus* isolate LSV-SL
	Palampur	AJ781318	*Lily symptomless virus* isolate LSV-Lt
	Palampur	AJ812569	*Chrysanthemum virus* B
	Palampur	AJ812733	*Chrysanthemum virus* B isolate Dehradoon
	Guwahati	AJ812735	*Chrysanthemum virus* B
	Palampur	AJ831415	*Lily symptomless virus* isolate LSV-A
	Palampur	AJ831416	*Lily symptomless virus* isolate LSV-A
	Palampur	AJ831417	*Lily symptomless virus* isolate LSV-A3
	Chamba (H. P.)	AJ871365	*Chrysanthemum virus* B
	Srinagar	AJ871366	*Chrysanthemum virus* B
	Leh	AJ871367	*Chrysanthemum virus* B
	Gujarat	AJ871582	*Chrysanthemum virus* B
	Orissa	AJ879077	*Chrysanthemum virus* B
	Uttar Kashi	AJ879078	*Chrysanthemum virus* B
	Hisar	AM039440	*Chrysanthemum virus* B
	Kerala	AM039441	*Chrysanthemum virus* B
	Chattisgarh	AM039442	*Chrysanthemum virus* B
	Palampur	AM087400	*Lily symptomless virus* isolate Palampur
	Palampur	AM087401	*Lily symptomless virus* isolate Tulip
	Palampur	AM087402	*Lily symptomless virus* isolate Tulip

Japan		AB004567	*Garlic latent virus clone* GNAG-9C2
		AB004684	*Garlic latent virus clone* GCHI- 3C1
		AB004685	*Garlic latent virus clone* GUAE- 13C1
		AB004686	*Garlic latent virus clone* GI-3C2
		AB004802	*Shallot latent virus* clone SI-4C2
		AB004803	*Shallot latent virus* clone LI-3C1
South Korea		AJ971442	*Daphne virus* S isolate Kr4
		AJ971465	*Daphne virus* S isolate Kr5
		AJ971466	*Daphne virus* S isolate Kr6
		AJ971467	*Daphne virus* S isolate Kr7
		AJ971468	*Daphne virus* S isolate Kr8
		AJ971469	*Daphne virus* S isolate Kr9
		AJ971470	*Daphne virus* S isolate Kr10
		AJ971471	*Daphne virus* S isolate Kr11
		AJ971472	*Daphne virus* S isolate Kr12
		AJ972376	*Daphne virus* S isolate Kr3
		DQ520092	*Garlic common latent virus* isolate K2
		DQ520093	*Garlic latent virus* clone GLV-K12
USA		AF024628	*Cowpea mild mottle virus* CPMMV-H
		AF024629	*Cowpea mild mottle virus* CPMMV-M

Table 1: Description of 79 carlaviral coat protein gene sequences obtained from NCBI database.

Country	Alignment length (residues)	Identity (*) (residues)	Strongly similar (:) (residues)	Weakly similar (.) (residues)	Different (residues)
*Brazil and USA	2518	258 (10.25%)	0 (0.00%)	0 (0.00%)	2260 (89.75%)
Canada	919	439 (47.77%)	0 (0.00%)	0 (0.00%)	480 (52.23%)
China	926	265 (28.62%)	0 (0.00%)	0 (0.00%)	661 (71.38%)
India	986	259 (26.275)	0 (0.00%)	0 (0.00%)	727 (73.73%)
Japan	1034	345 (33.37%)	0 (0.00%)	0 (0.00%)	689 (66.63%)
South Korea	975	219 (22.46%)	0 (0.00%)	0 (0.00%)	756 (77.54%)

Table 2: Carlaviral coat protein nucleotide sequence alignment data generated for six countries by ClustalW program of Pole Bioinformatique Lyonnais software.

Country	Alignment length (residues)	Identity (*) (residues)	Strongly similar (:) (residues)	Weakly similar (.) (residues)	Different (residues)
Brazil and USA	853	9 (1.06%)	45 (5.28%)	45 (5.28%)	754 (88.39%)
Canada	304	133 (43.75%)	66 (21.71%)	31 (10.20%)	74 (24.34%)
China	312	79 (25.32%)	60 (19.23%)	36 (11.54%)	137 (43.91%)
India	328	39 (11.89)	43 (13.11%)	33 (10.06%)	213 (64.94%)
Japan	350	11 (3.14%)	25 (7.14%)	34 (9.71%)	280 (80.00%)
South-Korea	322	67 (20.81%)	45 (13.98%)	34 (10.56%)	176 (54.66%)

Table 3: Carlaviral coat protein amino acid sequence alignment data generated for six countries by ClustalW program of Pole Bioinformatique Lyonnais software.

```
                 2095      2105      2115      2125      2135      2145      2155      2165      2175      2185      2195       2205      2215
                    |         |         |         |         |         |         |         |         |         |         |          |         |
AF0246291   GAAGTGATGCTAGGCTACAAGAAAGTTGCTATTTTATTGTGCTTGTGTGTTAATAAAAATGGTTTTGAGCTGCCATTTGAGTTGTGTGTCTTGATAGCTCTAAAGTCTGAGCCCACACTAATTGGTCAAGGACG ATCCT
AF0246281   AAAGTAATGCTGGGTTACAAGCGACTGTCTGTTCTTCTCTATTTGTGTTCAAAT AAAATAGGTTGTTGTTTGCCGTTTGATTTGTGTGTTTTAATAGCTTTGAAGTGTGATCCTTCTAAAATAAATTATGGTGA GTCTT
AJ7482771   TAATGGATGA---------------------------------------------------------------------------------------------------------------------------- -----
AF2284161   CAATGCAGACTAG------------------------------------------------------------------------------------------------------------------------- -----
             **   *
Prim.cons.  4AA22GATGCTAGG2TACAAG22A2T22CT2TT2T22T2T22TTGTGT222AATAAAA22GGTT2T2222TGCC2TTTGA2TTGTGTGT2TT2ATAGCT2T2AAGT2TGA2CC2 2C222AAT222T2A2GG2222TC2T
```

Figure 1: Region of multiple sequence alignment of CP nucleotide sequence of *Lily symptomless virus* isolate LSV-Oh with that of other carlaviruses from Brazil and USA showing AATAAA motif found in the alignment.

test LSV sequence at nucleotide and amino acid levels, respectively, which indicated high level of identities within the Indian CVB isolates coat protein sequences. In earlier studies similar higher level of identities were obtained among the CP gene sequences of Indian CVB isolates, ranging from 74-98% and 74-99% at nucleotide and amino acid levels, respectively (Singh et al., 2007).

AATAAA (Polyadenylation signal motif) was searched for in the six multiple nucleotide sequence alignments of CP gene sequences of carlaviruses but it was not conserved in any of the LSV isolates under study. AATAAA motif was found completely conserved in two CPMMV isolates from USA (CPMMV-H; AF024628 and CPMMV-M; AF024629; Figure 1) along with 7 HpLV isolates (EF202598, EF202599, EF202600, EF394781, EF394782, EF394783 and EF394784) from China (Figure 2) and 3 DVS isolates (AJ971469, AJ971470 and AJ971471) from South Korea (Figure 3). This motif is also found in other viruses such as *Rice tungro bacilliform virus* (RTBV) belonging to caulimoviridae family, where it forms an essential part of the poly (A) signal (Rothnie et al., 2001) and in 3´ untranslated region of ORF5 of *Banana mild mosaic virus* (genus not assigned) (Gambley and Thomas, 2001). The AATAAA nucleotide motif was absent from multiple sequence alignment of Canada and India.

Six phylogenetic trees of carlaviral CP nucleotide sequences prepared by ML method for different countries placed the test LSV closest to GarCLV isolates from Brazil (Figure-4A and Figure-4B), PVM

Journal of Proteomics & Bioinformatics - Open Access

Research Article
OPEN ACCESS Freely available online
doi:10.4172/jpb.1000141

JPB/Vol.3 Issue 6

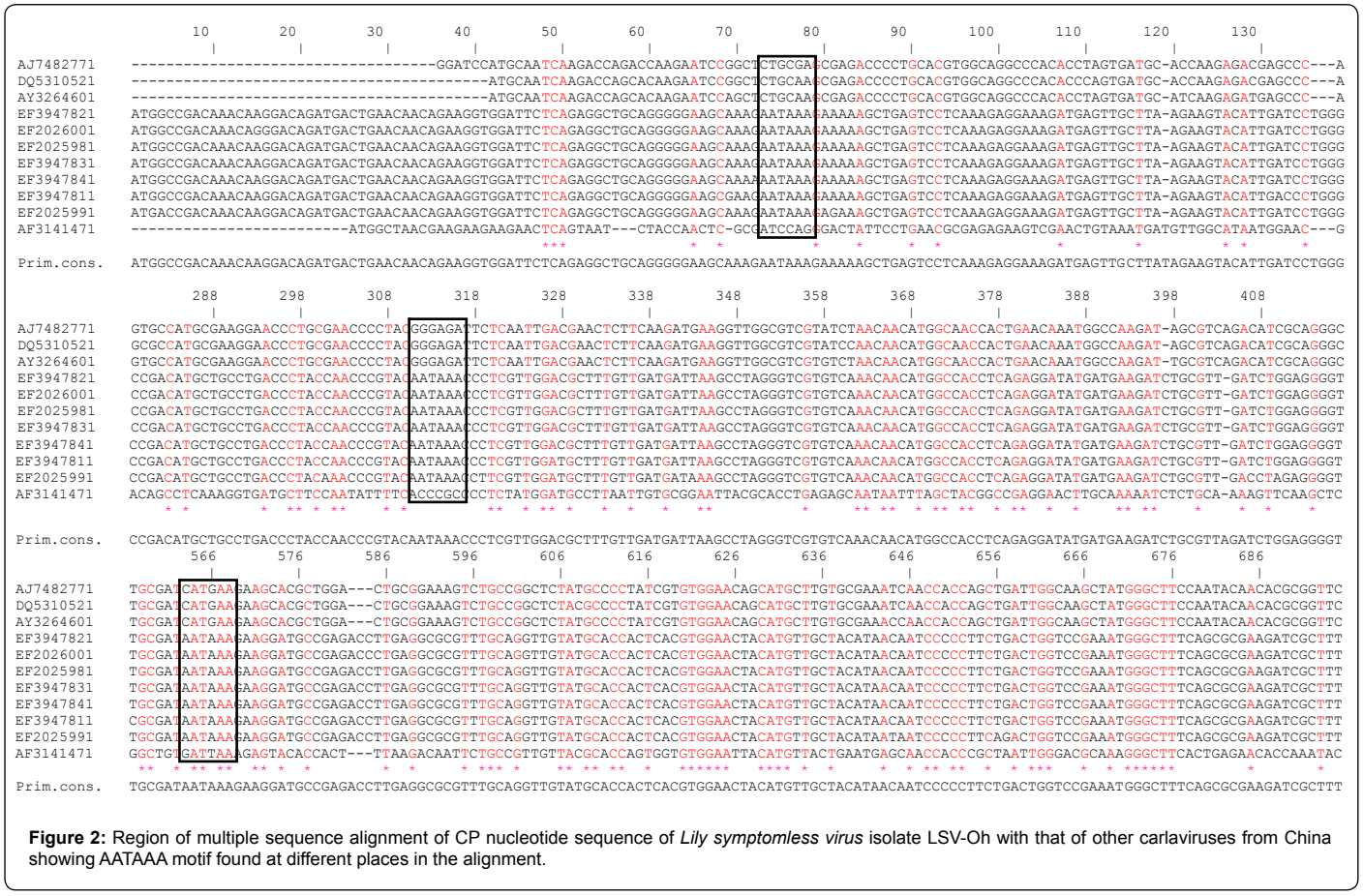

Figure 2: Region of multiple sequence alignment of CP nucleotide sequence of *Lily symptomless virus* isolate LSV-Oh with that of other carlaviruses from China showing AATAAA motif found at different places in the alignment.

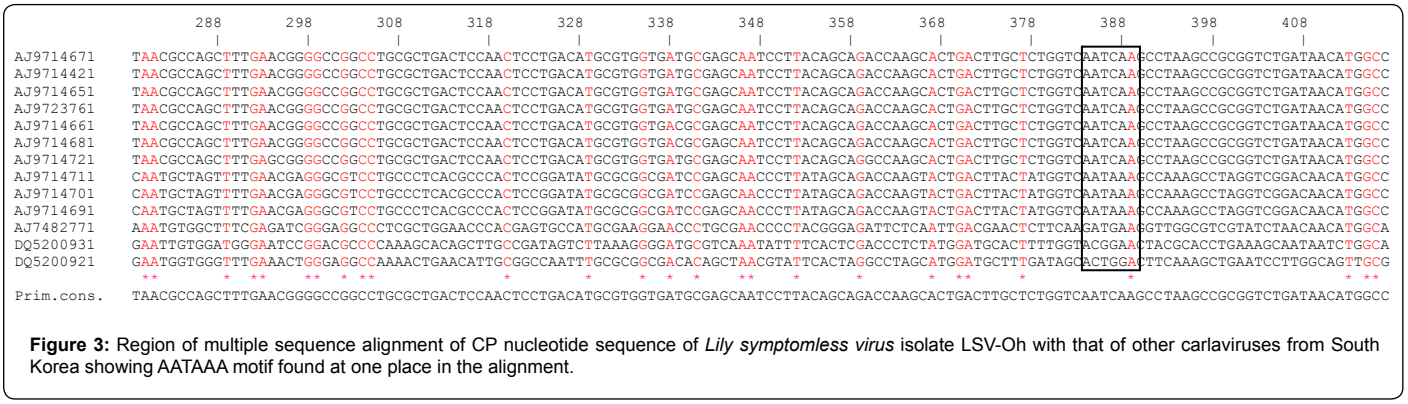

Figure 3: Region of multiple sequence alignment of CP nucleotide sequence of *Lily symptomless virus* isolate LSV-Oh with that of other carlaviruses from South Korea showing AATAAA motif found at one place in the alignment.

isolate Ca 513 from Canada (Figure-4C and Figure-4D), LSV isolate Yunnan from China (Figure-4E and Figure-4F), LSV isolate Palampur from India (Figure-4G), SLV clone LI-3C1 from Japan (Figure-4H and Figure-4I) and GarLV clone GLV-K12 from South Korea (Figure-4J and Figure-4K).

A combined phylogenetic tree prepared by NJ algorithm using all carlaviral sequences revealed that at nucleotide level, all the LSV isolates studied under investigations formed a single big cluster and the test LSV was placed closest to the LSV isolate Palampur (AJ585052) from India, followed by LSV isolates Yunnan (AY326460) and Lanzhou (DQ531052) from China (Figure 5). Similarly, combined phylogenetic tree prepared for all carlaviral coat protein amino acid sequences showed that test LSV shared its most recent common ancestry with the LSV isolates from Palampur India and LSV isolates from China (Figure 6).

Earlier studies revealed that CP sequences of Indian LSV isolate forms a cluster with one of the LSV isolate from China (Singh et al., 2005); similarly, our study also placed the LSV sequences from China (AY326460 and DQ531052) in a cluster of Indian LSV isolates. But an important finding in our studies of the combined phylogenetic analysis with carlaviral sequences of the world using NJ method is that at nucleotide level LSV isolates, Yunnan; AY326460 and Lanzhou; DQ531052, from China are placed quite near to the test sequence (only after LSV isolate Palampur; Figure 5) but at amino acid level (Figure 6) they are placed a little farther from the test LSV (placing 5 LSV isolates from Palampur (H.P.) in between them) which indicated that there is a functional variation in these viruses as depicted by the analysis at amino acid level, whereas at nucleotide level these viral sequences from H.P. (India) and China depicted significant similarities.

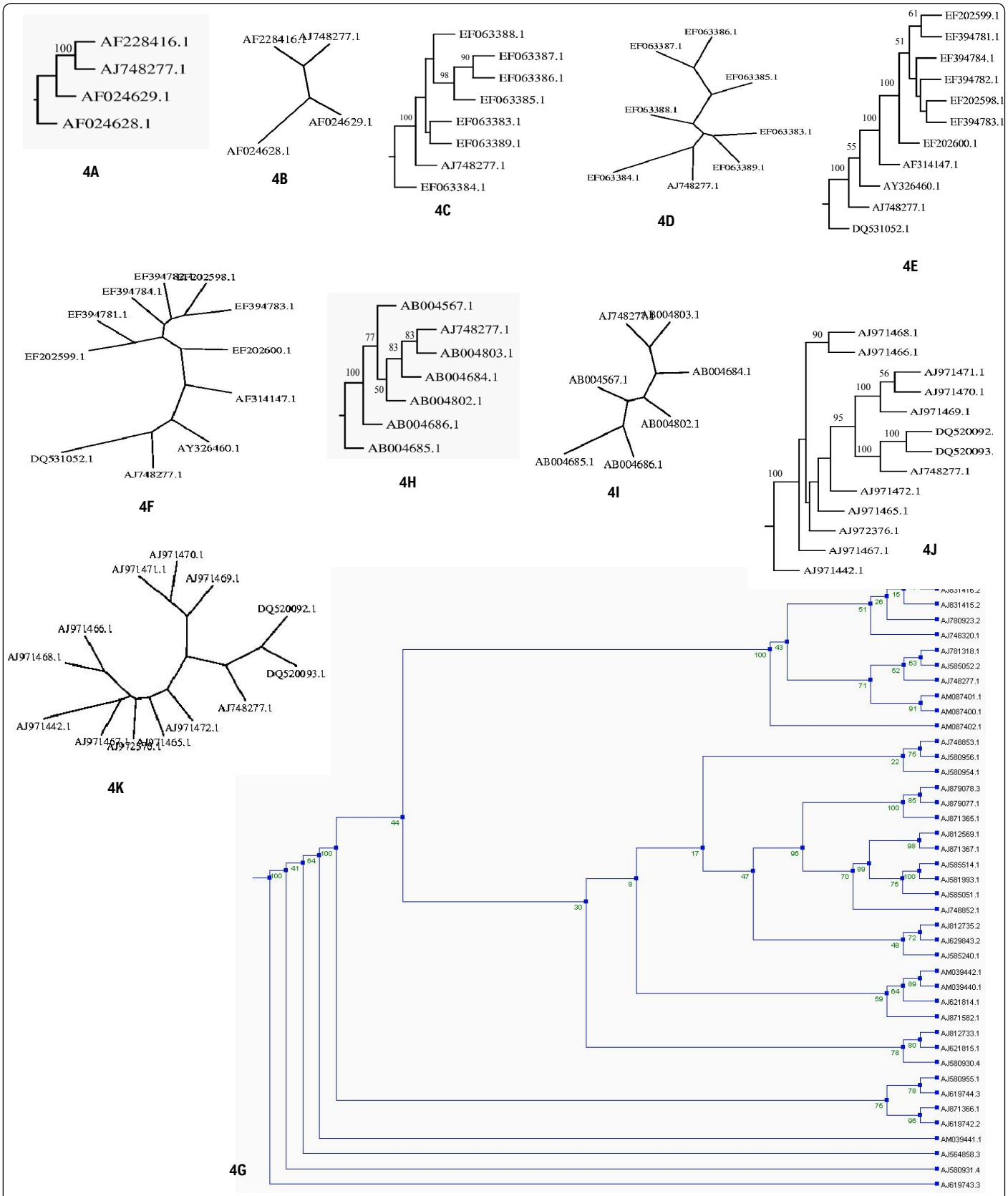

Figure 4: Rooted (DRAWGRAM) and unrooted (DRAWTREE) Maximum Likelihood trees showing phylogenetic relationship of *Lily symptomless virus* isolate LSV-Oh with the members of genus *Carlavirus* from Brazil and USA (A and B), Canada (C and D), China (E and F), India (G), Japan (H and I) and South korea (J and K), based on CP gene nucleotide sequences. Numbers at each node of the trees indicate the percentage of bootstrap samples (100 replications) (only values ≥ 50 are shown).

Research Article
OPEN ACCESS Freely available online
doi:10.4172/jpb.1000141
JPB/Vol.3 Issue 6

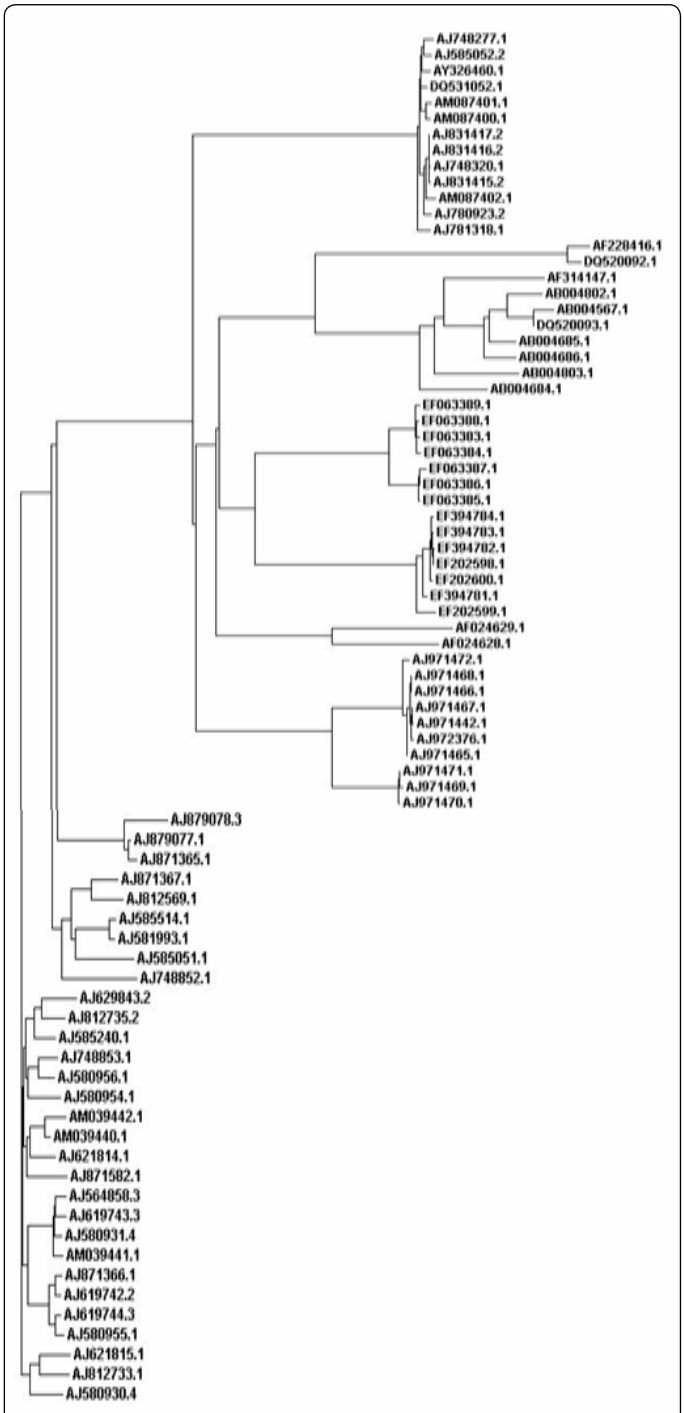

Figure 5: Neighbor-joining tree showing relationship between *Lily symptomless virus* isolate LSV-Oh and members of the genus *Carlavirus* from around the world present in NCBI database based on CP gene nucleotide sequences.

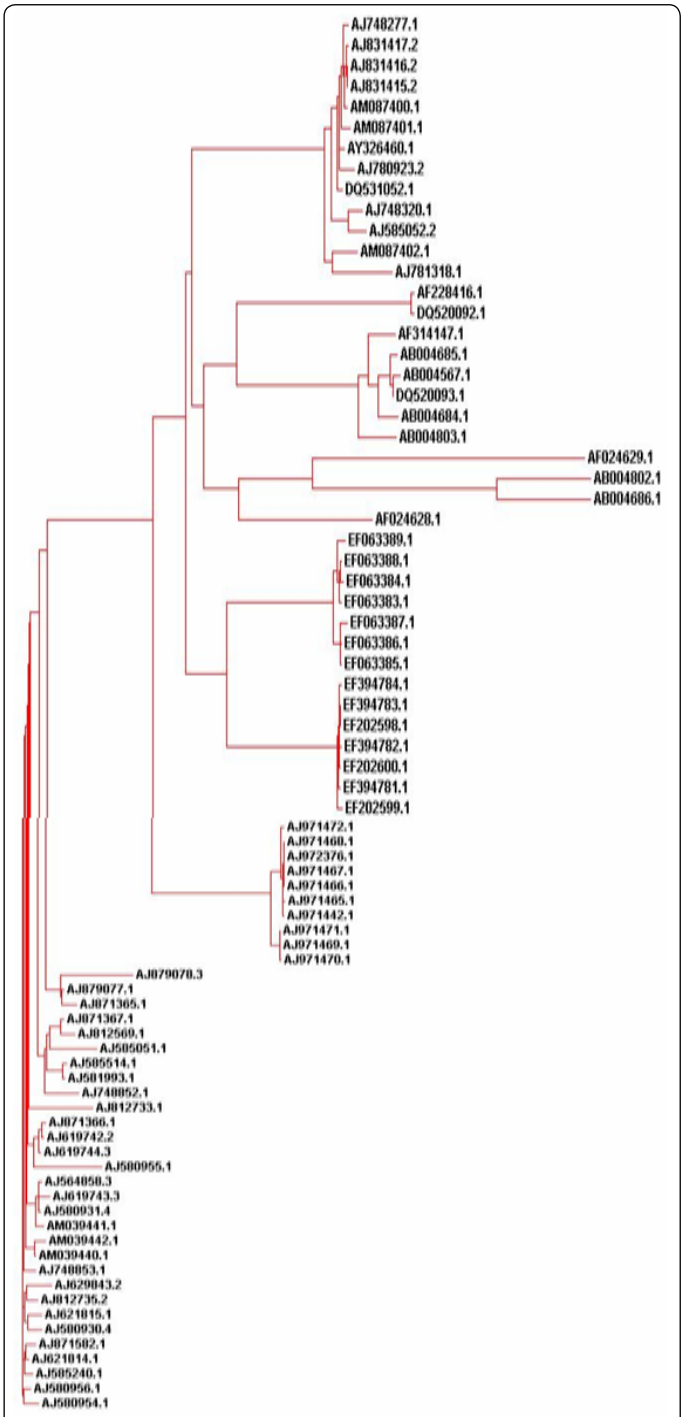

Figure 6: Neighbor-joining tree showing relationship between *Lily symptomless virus* isolate LSV-Oh and members of the genus *Carlavirus* from around the world present in NCBI database based on CP gene amino acid sequences.

This may be attributed to the changes in LSV sequences of either India or China at translational level due to microenvironment of the virus depending upon the climatic factors and virus-host interactions in the respective countries of their evolution.

Conclusions

On the basis of multiple sequence alignment, motif studies, and phylogenetic analysis it could be interpreted that in *Lily symptomless virus* variations are taking place at a faster pace at nucleotide level. Although at present much of the functioning of the coat protein gene have not been halted but if these variations keep on accumulating then (at sudden point) it may lead to evolution of new strains of viruses capable of widespread dispersal and damage. Also, this virus shared its most recent common ancestry with its native LSV isolates from India and with LSV isolates from China, probably indicating its origin in either of the countries. However, extensive experimentation is required to study the real consequences of these changes or variations occurring in the coat protein of LSV at nucleotide level, especially in its conserved motif sequences.

Acknowledgements

The authors are thankful to the Department of Biotechnology, Government of India and BTIS - SubDIC, Dr Y.S. Parmar University of Horticulture and Forestry, Nauni, Solan (H.P.), India for financial and technical support during these investigations.

References

1. Adams MJ, Antoniw JF, Bar-Joseph M, Brunt AA, Candresse T, et al. (2004) The new plant virus family *Flexiviridae* and assessment of molecular criteria for species demarcation. Arch Virol 149: 1045-1060.
2. Allen TC (1972) Lily symptomless virus. *CMI/ABB Descriptions of Plant Viruses*. Wellesbourne, UK: Association of Applied Biologists 96: 4.
3. Allen TC (1975) Viruses of lilies and their control. Acta Hort 47: 69-75.
4. Asjes CJ (1998) Data sheet on *lily symptomless carlavirus*. In *CABI Crop Protection Compendium*. Wallingford, UK: CAB International.
5. Asjes CJ (2000) Control of aphid- borne lily symptomless virus and lily mottle virus in Lilium in the Netherlands. Virus Research 71: 23-32.
6. Brierley P, Smith FF (1944a) Studies on lily virus diseases: the necrotic fleck complex of *Lilium longiflorum*. Phytopathol 34: 529-555.
7. Brierley P, Smith FF (1944b) Studies on lily virus disease: the mottle group. Phytopathol 34: 718-746.
8. Brierley P, Smith FF (1945) Additional specimens of *Lilium* susceptible of *lily rosette virus*. Phytopathol 35: 129-131.
9. Brunt AA, Foster GD, Morozov SY, Zavriev SK (2000) Genus carlavirus. In *Virus taxonomy: classification and nomenclature of viruses, seventh report of the International Committee on Taxonomy of Viruses*. Academic Press 969-975.
10. Cavileer TD, Halpern BT, Lawrence DM, Podleckis EV, Martin RR, et al. (1994) Nucleotide sequence of the carlavirus associated with blueberry scorch and similar diseases. J Gen Virol 75: 711-720.
11. Derks AFLM (1995) Bulbs and corm crops. In: *Virus and virus-like disease of bulbs and flower crops*. Edited by Loebenstein G, Lawson RH, Brunt AA. John Wliey and Sons, New York 541.
12. Derks AFLM, Asjes CJ (1975) Lily symptomless virus in tulip. Netherlands. Eur J Plant Pathol 81: 14-21.
13. Fuji S, Yamamoto H, Inoue M, Yamashita K, Fukui Y, et al. (2002) Complete nucleotide sequence of the genomic RNA of Aconitum latent virus (genus carlavirus) isolated from Delphinium sp. Arch Virol 147: 865-870.
14. Gambley CF, Thomas JE (2001) Molecular characterization of banana mild mosaic virus, a new filamentous virus in *Musa spp*. Arch Virol 146: 1369-1379.
15. Hataya T, Uchino K, Arimoto R, Suda N, Sano T, et al. (2000) Molecular characterization of hop latent virus and phylogenetic relationships among viruses closely related to carlaviruses. Arch Virol 145: 2503-2524.
16. Lee BY, Choi SH, Ryu KH (2003) Characterization of the 3´-terminal nucleotide sequence of two Korean isolates of Daphne virus S support its placement as a distinct species of the genus Carlavirus. Arch Virol 148: 1915-1924.
17. Lee KH (1992) Disease incidence, symptomatology and identification of two new isometric viruses from imported lilies. MSc thesis. Chunnam National University. Gwangju, Republic of Korea.
18. Pearson WR (2000) Flexible sequence similarity searching with the Fasta3 program. Methods Mol Bio 132: 185-219.
19. Rothnie HM, Chen G, Futterer J, Hohn T (2001) Polyadenylation in rice tungro bacilliform virus: cis-acting signals and regulation. J Virol 75: 4184-4194.
20. Singh AK, Hallan V, Ram R, Zaidi AA (2005) Variability in the coat protein of *lily symptomless virus* isolates infecting various lily species. Plant Pathol 54: 621-624.
21. Singh L, Hallan V, Jabeen N, Singh AK, Ram R, et al. (2007) Coat protein gene diversity among chrysanthemum virus B isolates from India. Arch Virol 152: 405-413.
22. Zavriev SK, Kanyuka KV, Levay KE (1991) The genome organization of potato virus M RNA. J Gen Virol 72: 9-14.