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Original Article

The Major Mechanisms of Genetic Differentiation Among Apricot Latent Virus (ApLV) Isolates

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Abstract

Background and Aims: Apricot latent virus (ApLV) is a species within Foveavirus genus (Betaflexiviridae family, Tymovirales order). Phylogenetic analyses using different ORFs nucleotide sequences divided most ApLV isolates into two clusters. However, there is little data about the sources of genetic differentiation among ApLV isolates.

Materials and Methods: Partial coat protein (CP) sequences of two Iranian ApLV isolates were determined. The complete or partial CP sequences of ApLV isolates available in the GenBank with two partial CP sequences of Iranian isolates were used for phylogenetic analyses. Also, non-overlapping regions of ORFs were considered for molecular analysis using PATRISTIC, MEGAX, and Phylip 3.67 softwares.

Results: High nucleotide diversity 0.0 to 39.2 % was observed for TGB2 gene, followed by CP 0.0 to 30.9 %, RdRp 0.0 to 30.3% and TGB1 0.0 to 25.9 % genes. Although Caserta12 and SB12452 isolates showed the low nucleotide diversity in RdRp, TGB1 and TGB2 genes, but high nucleotide diversity was detected for them in CP gene (26.7 to 30.9 %). Alignment of CP indicated insertion/deletion may affect the phylogenetic differentiation of ApLV isolates. Comparision of nucleotide diversity within and between groups showing that founder effect may affect the variation of ApLV isolates. The values of FST between ApLV phylogenetic groups were < 0.33 indicating a relative rate of dissociation. Recombination breakpoints were detected in the different ORFs especially in Caserta12 isolate from Italy. The ω ratios (dNS/dS) in various proteins were conserved from 0.035 to 0.073. The ENC plot analysis indicated that the codon usage is affected by selection instead of mutation pressure for all genes except TGB2 gene. In addition, the results of mean CAI values indicated the TGB2 gene as a high expression gene. The genetic exchanges by recombination were also correlated with the appearance of new ApLV strains. ENC-Plot and mean CAI analysis are evaluated for the first time in this study to better explains the evolutionary process and fitness of ApLV.

Conclusion: Altogether, our analysis indicated that recombination, mutation and selection pressure are the major sources of genetic differentiation among ApLV isolates.

Keywords: *Apricot latent virus* (ApLV); genetic analysis; Recombination

Introduction

Prunus is a genus of trees and shrubs, which includes the plum (Prunus domestica), peach (P. persica), apricot (P. armeniaca), cherry (P. avium) and sour cherry (P. cerasus). The wide diversity of species in the Prunus genus is reflected in a large number of viruses causing disease to those plants (1).

At least they can be infected with more than 25 graft-transmissible diseases (2). Stone fruits are natural hosts of different viruses such as Ilarvirus, Potyvirus and Trichovirus genus.

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One of the other families of viruses infecting these species are even some representatives of the genus Foveavirus, which expansion and economic importance for fruit growing is still not fully known (2, 3). Apricot latent virus (ApLV) is a decisive member in the Foveavirus genus (Betaflexiviridae family, Tymovirales order) (4-6) and described for first time in asymptom apricot cv. Silistra in Moldova (7). ApLV also reported from Palestine (8), western Turkey (9), Iran (10), Italy (11) and France (12), Egypt (13), Lebanon (14), Spain (15), and Czech Republic (3).

Narrow host range has been reported for ApLV (4). Apricot trees infected by this virus did not elicit visible symptoms with the exception of malformation and chlorotic blotching of the new leaves and shoots in some cultivars (3, 16). Recently using deep sequencing ApLV infection was identified on apple tree (Malus pumila) (17). ApLV is consideted as a putative factor of sooty ringspots or yellow asteroid on the leaves of graft-inoculated peach seedlings (7, 12, 18-21). Chlorotic spots, mottling and red to purple rings were also occasionally observed on the leaves of graft-inoculated P. cerasifera (22) and P. avium (8), respectively. Grafting is the main manner for transmission of ApLV and it survives in the propagative materials .

Stone fruit cultivation in Iran has a long history. Iran is one of the most important stone fruit suppliers as it produces about two milion tons annually (23). Virus diseases are a limiting factor in the production of stone fruits in Iran. Leaf chlorosis, chlorotic spots, mottle, and/or mosaic, leaf distortions, stunting bark cracks, and stem pitting on tree trunks are symptoms commonly observed on fruit trees. In spite of the importance role of stone fruit viruses, limited information is available about these viruses in Iran. In order to access data on the molecular genetic diversity and population structure of ApLV the partial CP gene of two Iranian ApLV isolates was sequenced and analyzed with those of CP gene for the other ApLV isolates already reported.

Methods

Sampling: During growing seasons of 2016 and 2017, leaf samples were collected from apricot trees in different orchards that displayed stunting, chlorotic spots and/or mottle symptoms (Fig. 1a-c). The surveyed regions were included 17 orchards in Ardabil, Azarbayjan-e-Gharbi and Azarbayjan-e-Sharghi provinces in North-West Iran. Totally 99 leaf samles were collected and checked for the detection of ApLV by RT-PCR using specific primers.

Mechanical inoculation: Herbaceous indicator plants species belonging to the various families such as Amaranthaceae (Gomphrena globosa), Chenopodiaceae (Chenopodium quinoa, Ch. Amaranticolor), Cucurbitaceae (Cucumis sativus), Fabaceae (Vigna unguiculata), Lamia-ceae (Ocimum basilicum) and Solanaceae (Nicotiana occidentalis, N. benthamiana, N. glutinosa, N. tabacum cv. White Burley, N. tabacum cv. Xantia, N. tabacum cv. Samsung, N. rustica, Petunia hybrid were used for mechanicall inoculation by leaves from the symptomatic plants. A general inoculation buffer, 0.01 M K-phosphate buffer (pH 7.0) including 2% nicotine was used in mechanical transmission test. Totally six plants from each indicator species were inoculated and biological assay was iterated three times. The inoculated plants were preserved in an insect-free greenhouse at 25±5°C, 50 -70% relative humidity, and 15 hours light. The inoculated plants were regularly observed.

All asymptomatic and symptomatic plants were tested by RT-PCR four to five weeks post inoculation.

RNA extraction, RT-PCR and Sequencing: The total RNA was extracted from leaf samples by Tri-reagent (Sigma, USA). Partial CP sequences of nine ApLV isolates were amplified by RT-PCR using designed primers (20). Complementary DNA (cDNA) are synthesis in final 20 µL volume by mixing of 1 µL of reverse primer C-ALV1 (5'-AGCAAGGTAAACGCCAAC-3') (20 pmol/ µL), 2 µL of dNTPmix (10 mM), 2 µL of 10x first-strand buffer [250 mM Tris-HCl (pH 8.3), 375 mM KCl, 15 mM MgCl2], 2 µL of DTT

(0.1M), 1 µL of RNase inhibitor (40 U) and 200 U of M-MLV (Sinaclone, Iran). The tubes were heated at 25ºC for 10 min and then at 42ºC for 60 min. For the PCR, 5 µL of the cDNA was moved to a new tube and 5 µL of 10x PCR Buffer [200 mM Tris-HCl (pH 8.4), 500 mM KCl], 3 µL of MgCl2 (50 mM), 2 µL of dNTP mix (10 mM), 2 µL of primers H-ALV1 (5'-GGAATAGAGCCCCAAGAAG-3') (10 µM), 1 µL of Taq DNA polymerase (5 U) and 30 µL of DEPC-treated water was added. The cycling steps for PCR was 94ºC for 3 min followed by 35 cycles of 95ºC for 2 min, 50ºC for 90s and at 72ºC for 60s; and a final extension step at 72ºC for 10 min.

PCR amplicons were cloned into pGEM-T Easy vector (Promega, Madison, WA, USA) and applied to transform DH5α Escherichia coli competent cells according to the manufacturer's instructions. Using QIApreb Spin Miniprep Kit (QIAGEN, Valencia, CA, USA) the recombinant plasmids were extracted and cleaned for nucleotide sequencing. Sequence data were aligned using CLUS-TALX2 (24).

Phylogenetic tree and nuleotide distance: The compelete or partial CP sequences of ApLV isolates available in the GenBank with two partial CP sequences of Iranian isolates were used for phylogenetic analyses (Table 1). Non-overlapping regions of ORFs were considered for molecular analysis. Maximum-Likelihood (ML) method with Kimura's two-parameter (K2) plus gamma distribution (K2+G) in MEGAX was used for phylogenetic tree (25). PATRISTIC programe was used for ML trees comparison (26). Nucleotide distances and nucleotide diversity were determined by the maximum-composite-likelihood method with MEGAX (25). The ω ratios (dNS/dS) were computed by Pamilo-Bianchi-Li (PBL) method based on Kimura's two-parameter model (27). Phylip 3.67 software was considered for analysis of pairwise genetic distances (28). The frequencies of haplotype in the population were computed using DNASP version 4.10 (29).

Detection of recombination and selection analysis: Relationships between aligned genes (Table 1) were computed individually by ML method available in MEGAX (25). Several methods available in the RDP4 version 4.70 were used for detection of recombination events, recombination cross-over point, and parental isolates of recombinants (30) with default setting and Bonferroni corrected P-value cut-off of 0.01 and 0.05 .

Analysis of effective number of codons (ENC) and codon adaptation index (CAI): The codon usage variations among genes in various organisms can be represented by ENC plot (a plot of ENC vs GC3 content) (31). ENC-plot analysis was accomplished to investigate which one of the selection pressure or mutation pressure forces influenced the codon usage patterns in genes. In general, when the data points drop under the standard curve, the codon usage is affected by selection pressure rather than the mutation pressure, and vice versa. In addition, the codon adaptation index (CAI) values which reflect the gene expression level was determined. The CAI value ranges from 0.0 to 1.0 indicates the difference expressed genes. The highest mean CAI value shows the stronger codon usage bias and a higher expression (32-34). CodonW version 1.4.2 (John Peden, available at http://source-forge.net/projects/codonw/), an integrated program, was employed for CAI and ENC values

Results

Sampling and host range: In surveyed orchards, fruit trees were inspected for virus symptoms. Symptomatic trees showing chlorotic or necrotic spots and mottle were observed in stone fruit orchards. Totally eight (8.08%) out of 99 leaf samples were determined to be infected with ApLV by RT-PCR. Biological assay using different indicator plants confirmed only the infection of N. occidentalis, 20 to 30 days after inoculation (Fig. 1d). The infection of inoculated plants was confirmed by RT-PCR .

Sequence analysis and phylogenetic relation-ships: A PCR product with the approximately 200 bp was obtained for seven of eight RNA extracts from inoculated N.

occidentalis. The sizes of PCR products were similar.

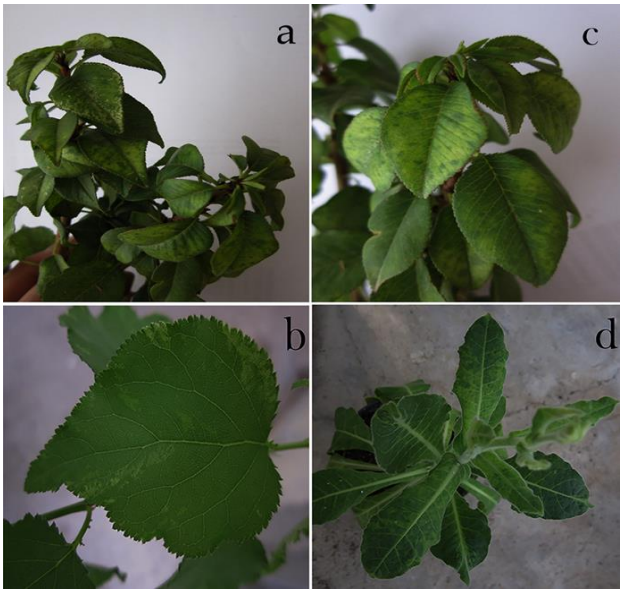

Fig. 1. (a, b, c) Apricot infected by Apricot latent virus. (d) Mottle and vein yellows symptoms on Nicotiana occidentalis inoculated with Iranian ApLV isolate.

Table 1. Apricot latent virus isolates analyzed in this study

Isolate/variant	Host/Cultivar	Location	genome region/ Ac. no.
A18	*Prunus armeniaca*/Sorrentina GF305	Italy	Full/HQ 339956
SB12452	*P. persica*/Neptune GF305	France	Full/HQ 339957
LA2	*P. armeniaca*/GF305	France	Full/HQ 339958
Caserta12	*P. armeniaca*/GF305	Italy	Full/HQ 339959
09PALpe1	*Prunus persica*/Missour	Italy	CP/FN25 2232
09PALap1	*P. armeniaca*/Tirynthos	Italy	CP/FN2 52234
09PALap2	*P. armeniaca*/Baracca	Italy	CP/FN2 52235
09PALpl1	*P. salicina*/Autumn Giant	Italy	CP/FN2 52236
09PALpl2	*P. salicina*/Ozark Premier	Italy	CP/FN2 52237
09PALche1	*P. avium*/Adriana	Italy	CP/FN2 52238
09MOL	*P. persica*/GF	Italy	CP/FN2 52239
09TUR	*Prunus spp.*/-	Turkey	CP/FN2 52240
Apr47	*P. armeniaca*/-	Palestinian	CP/AY6 97862
AprSp.mur1	*P. americana*/Murciana	Spain	CP/GQ9 19051
NemMD	*P. persica*/-	-	CP/AF0 57035
ApL-Iran1	*P. armeniaca*	Iran/This Study	CP/MW 044949
ApL-Iran2	*P. armeniaca*	Iran/This Study	CP/MW 044950

Therefore, the partial sequences of CP fragments of two isolates (ApL-Iran1 and ApL-Iran2) were obtained and deposited in GenBank with accession numbers MW044949 and MW044950, respectively. BLAST analysis and sequence alignment of CP nucleotide sequences revealed 97% nucleotide identity with ApLV Turkish isolates (accession no. FN252240). The phylogenetic trees based on all ORFs (Supplementary Fig. a-c, for RdRp, TGB1 and TGB2 respectively) indicted that ApLV isolates are separated into two clusters. Using all CP sequences (complete or partial sequences), ApLV isolates divided into clusters (Fig. 2) and Iranian ApLV isolates grouped in clusterI (Fig. 2). Two-dimensional pairwise nucleotide distance plot analysis also showed two phylogenetic clusters. High nucleotide diversity 0.0 to 39.2 % was indicated for TGB2 gene (Fig. S1), followed by CP (0.0 to 30.9 %) (Fig. 2) RdRp (0.0 to 30.3 %), and TGB1 (0.0 to 25.9 %) genes (Fig. S1). Although two Italian (Caserta12) and French isolates (SB12452) showed the low nucleotide diversity in RdRp, TGB1 and TGB2 genes (Fig. S1), but high nucleotide diversity was indicated for them in CP gene (26.7 to 30.9 %) (Fig. 2). The full-lengths of CP gene in Caserta12 and SB12452 isolates are 1215 and 1221 nucleotides respectively, in comparison to other isolates 1266 nucleotides. This variety of length for nucleotide sequences suggesting insertions or deletions.

Patristic distance plots: The maximum-likelihood trees of the individual ORFs were comparised by PATRISTIC approach. The pairwise plots of the distance in the trees deduced from the RdRp versus TGB1 (Fig. 3a) and RdRp vs. TGB2 (Fig. 3b) in which the two sets of distances shows a linear correlation coefficient of 0.984 and 0.830 ($p<0.001$), respectively. The plot of the TGB1 distances against TGB2 with a linear correlation coefficient of 0.908 (Fig. 3c) showed two distinct populations. Between CP and other ORFs no correlation was found.

Recombination analysis: The analysis of reco-mbination using various methods indicated that

the Caserta12 sequence had three recombination sites around nucleotides 1347 to 1435 in RdRp, 6820 to 7040 in TGB1 and 8542 to 8660 in CP genes (Table 2, Fig. 4b).

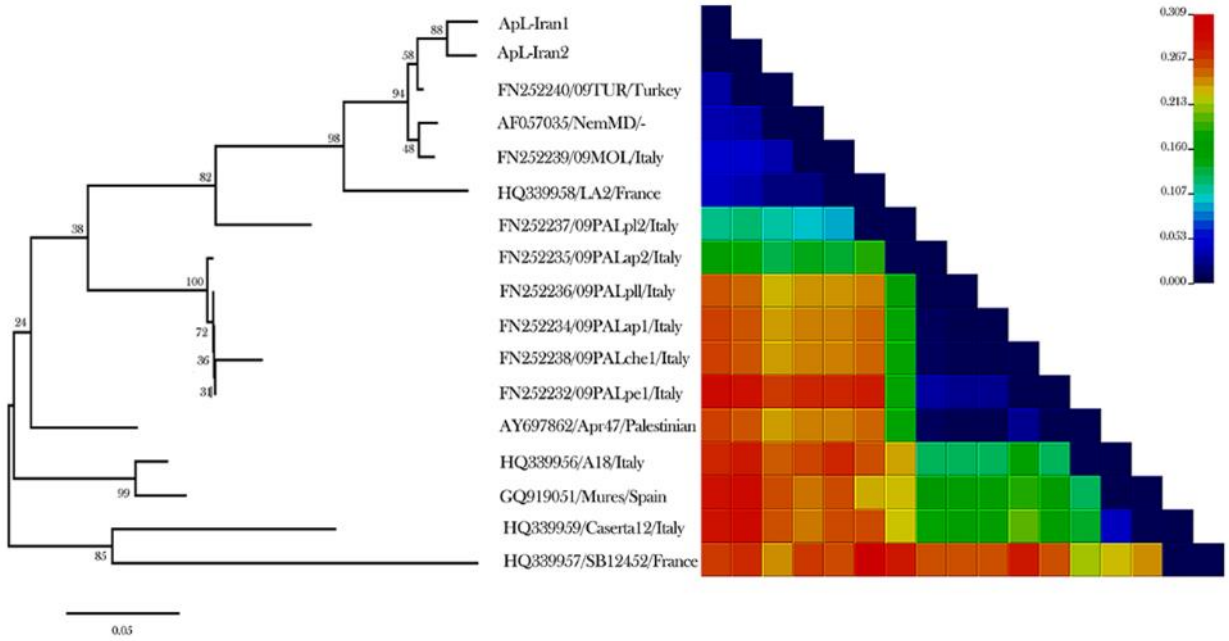

Fig. 2. Maximum Likelihood (ML) tree and two dimensional of nucleotide diversity plot showing the relationship among Apricot latent virus (ApLV) isolates. The tree was constructed using seventeen CP nucleotide sequences of ApLV isolates. Numbers at each node indicates the percentage of supporting puzzling steps (or bootstrap samples) in ML method. The name of each isolate and the country of its origin are listed in the accession number in the International Gene Sequence Database (GenBank).

This is an inter-recombinant of Caserta12 isolate in cluster-II with putative parents from cluster-I and cluster-II. By P-values using the Siscan programs of the RDP4 software the recombination sites were determined (Table 2). In addition, the potential of recombination sites were found in TGB1 for LA2 isolate (accession no. HQ339958), in TGB2 for A18 isolate (accession no. HQ339956) (Fig. 4c) and in CP for SB12452 isolate (accession no. HQ339957) (Table 2). However, the recombination breakpoints were not confirmed by multiple different methods and with a low related P-value for each of the methods.

Mean nucleotide diversity and ω ratio: The mean nucleotide diversities for the RdRp, TGB1, TGB2 and CP were 0.276, 0.224, 0.276 and 0.141, respectively (Table 3).

Additionally, the within-group diversity of ApLV genes was from 0.167 to 0.265 (Table 3). The dNS/dS ratios were estimated by the PBL method (27). The lowest and highest dNS/dS ratios were 0.035 and 0.073 for TGB1 and TGB2, respectively. Generally, the evolutionary restiction logged on TGB1 gene is greater than the one exerted on the other genes (Table 3).

Analysis of ENC-Plot and CAI: ENC plot analysis was accomplished to investigate which one of the selection pressure or mutation pressure forces influenced the codon usage patterns in ApLV genes.

By this plot the data point's clustered together under the standard ENC curve except for TGB2 gene (Fig. 5). When the data points drop under the standard curve, the codon usage is affected by selection pressure rather than the mutation pressure, and vice versa. In addition, the results of mean CAI values for different ApLV genes indicated the TGB2 gene as a high expression gene.

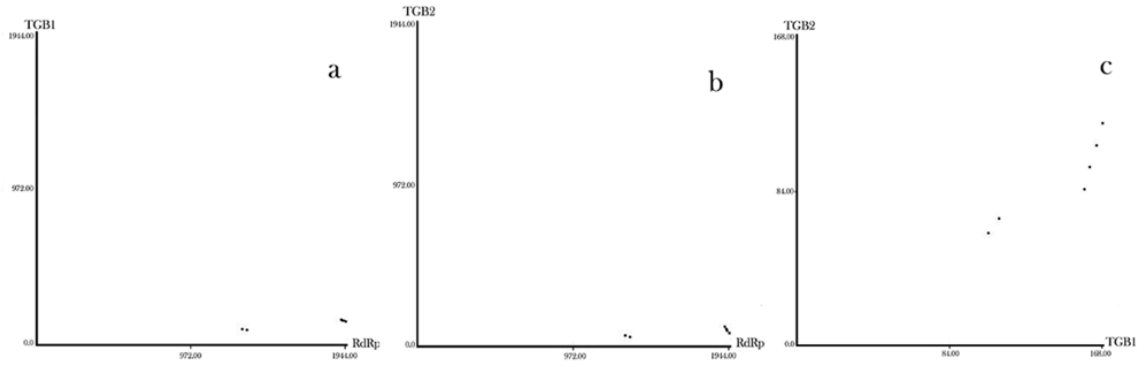

Fig. 3. Multidimensional scaling of tree-to-tree patristic distances (a) TGB1 vs RdRp isolates; (b) TGB2 vs RdRp isolates; and (c) TGB1 vs TGB2 isolates.

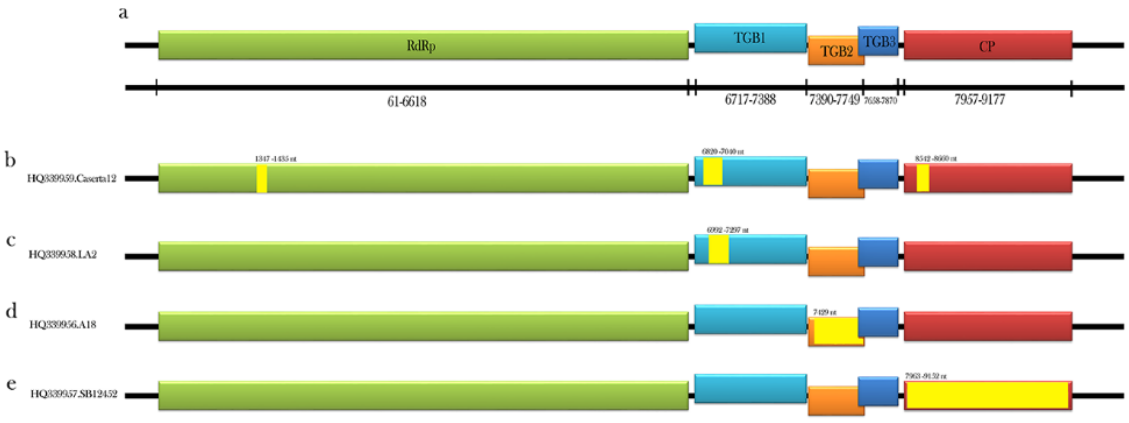

Fig. 4. The schematic recombination sites are shown for different genes of Apricoat latent virus using RDP4. The potential recombination sites were indicated by yellow box

Table 2. Crossover sites in Apricot latent virus isolates detected using recombination detecting program

ORFs	Recombinant (Ac. No)	Parental isolates (Ac. No./Simlartiy%) [a]		Breakpoints [b] /ORF		Methods [c]					
		Major parent	Minor parent	Begin	End	RDP	GENECONV	BootScan	Maxchi	Chimaera	Siscan
RdRp	Caserta12 (HQ339959)	SB12452 (HQ339957/80.7%)	LA2 (HQ339958/88.8%)	1347	1435	0.871	ND	3.365×10^{-2}	3.045×10^{-2}	1.183×10^{-1}	3.502×10^{-2}
TGB1	Caserta12 (HQ339959)	A18 (HQ339956/85%)	SB12452 (HQ339957/86.2%)	6820	7040	0.871	ND	5.865×10^{-2}	3.124×10^{-2}	1.307×10^{-2}	3.361×10^{-7}
	LA2 (HQ339958)	SB12452 (HQ339957/82.6%)	Unknown	6992	7297	0.739	ND	8.611×10^{-1}	5.641×10^{-2}	4.557×10^{-3}	1.779×10^{-6}
TGB2	A18 (HQ339956)	SB12452 (HQ339957/83.2%)	LA2 (HQ339958/83.2%)	7429	ND	0.892	1.452×10^{-1}	2.709×10^{-1}	2.240×10^{-1}	5.846×10^{-3}	3.667×10^{-7}
CP	Caserta12 (HQ339959)	Unknown	A18 (HQ339956/88.8%)	8542	8660	3.089×10^{-2}	ND	9.938×10^{-3}	4.263×10^{-1}	7.535×10^{-1}	3.832×10^{-4}
	SB12452 (HQ339957)	NemMD (AF057035/69.7%)	Unknown	7963	9152	0.796	ND	ND	3.251×10^{-1}	ND	3.495×10^{-5}

Table 3. Nucleotide and haplotype diversity of four ORFs of Apricot latent virus isolates [a]

ORFs	Phylogenetic groups	D[b]	Haplotype diversity	d_S	d_{NS}	d_{NS}/d_S
RdRp	Cluster I	0.231 (0.005)	1.000	1.107 (0.059)	0.053 (0.004)	0.047
	Cluster II	0.237 (0.007)	1.000	1.093 (0.053)	0.059 (0.004)	0.053
	All isolates	0.276 (0.006)		1.455 (0.045)	0.085 (0.004)	0.058
TGB1	Cluster I	0.181 (0.018)	1.000	0.906 (0.139)	0.009 (0.005)	0.009
	Cluster II	0.192 (0.018)	1.000	0.892 (0.143)	0.030 (0.010)	0.033
	All isolates	0.224 (0.015)		1.236 (0.135)	0.044 (0.008)	0.035
TGB2	Cluster I	0.194 (0.027)	1.000	0.721 (0.212)	0.052 (0.019)	0.072
	Cluster II	0.233 (0.029)	1.000	1.183 (0.516)	0.044 (0.022)	0.037
	All isolates	0.276 (0.024)		1.136 (0.153)	0.083 (0.020)	0.073
CP	Cluster I	0.167 (0.022)	0.9714	0.778 (0.124)	0.040 (0.011)	0.051
	Cluster II	0.265 (0.045)	1.0000	1.432 (0.715)	0.049 (0.028)	0.034
	All isolates	0.141 (0.012)		0.934 (0.106)	0.047 (0.013)	0.050

[a] Substitutions: *dNS* = average number of nonsynonymous substitutions per nonsynonymous site, *dS* = average number of synonymous substitutions per synonymous site, and *dNS/dS* = average of the ratio between nonsynonymous and synonymous substitutions.

[b] *D* = nucleotide diversity: average number of nucleotide substitutions per site between all pairs of sequences in the group. Standard errors are indicated in parenthes

Discussion

The aim of this study was to better understand the genetic structure and sequence diversity of ApLV population using various approaches. Stone fruit trees are considered as hosts for a lot of number of common RNA and economically important plant viruses from different genera. Howbeit ApLV is not highly prevalent; it was detected in countries including Iran thus far (3). In surveyed orchards, apricoat fruit trees were inspected for virus symptoms. Symptomatic trees showing chlorotic or necrotic spots and mottle (Fig. 1a-c) were observed. Totally eight (8.08%) out of 99 leaf samples were found to be infected with ApLV by RT-PCR. Biological assay using different indicator plants confirmed only the infection of N. occidentalis, 20 to 30 days after inoculation. The trials of mechanical transmission indicated that positive isolates had the same biological specifications with those of ApLV isolates previously reported (35) (Grimová and Ryšánek., 2012). Generally the infection of ApLV is symptomless therefore in the absence of a natural vector, infected but asymptom cultivars of apricot, cherry and plum makes a reservoir of virus and causes spread of ApLV through nursery productions. This should be taken into serious consideration in plant sanitary certification programs in order to harness, or decrease the potential contamination of the stocks with this virus (35).

Phylogenetic analysis using different ORFs grouped the ApLV isolates into two clusters (Fig. S1). Using CP gene most of the ApLV isolates were fell in phylogroup I. However, two French (SB12452) and Italian (Caserta12) isolates were far from other isolates and grouped in clusterII with high nucleotide distance 0.267 to 0.307 (Fig. 2). In addition, the full-lengths of CP gene in Caserta12 and SB12452 isolates are 1215 and 1221 nucleotides respectively, in comparision to other isolates with 1266 nucleotides. This variety of length for nucleotide sequences suggesting insertions or deletions. The PATRISTIC plots using ML trees also showed that the ORFs were closely linked and indicated similar evolutionary type (Fig. 3a-c).

Recombination is considered as one of the main sources of varaition in plant viruses by reducing mutational load, generation new viruses and reducing genetic differentiation (36). Three recombination loci at different genomic positions were detected for Italian isolate Caserta12 (Table 2, Fig. 4). However, high degrees of confidence were not indicated for these recombination events .

Different statistical tests were performed to access the evolutionary forces which affected the ApLV population. The FST program is used to determine the overall genetic differentiation between subpopulations. The complete sharing of genetic sequences and populations completely isolated from each other is indicated by FST 0.0 and 1.0, respectively (37).

The FST values (< 0.33) were determined among ApLV populations showing a relative level of separation. Pairwise FST comparisons between two clusters were 0.192, 0.208, 0.264 and 0.210, for RdRp, TGB1, TGB2 and CP genes, respectively. An absolute value of FST 0.33 suggests infrequent gene flow. A relative level of differentiation was indicated by the values of FST < 0.33 between ApLV phylogenetic groups .

Nucleotide and haplotype diversity values were also considered to estimate if ApLV population expansions have happened.

The mean nucleotide and the within-group diversities of each ApLV gene were similar to the other plant viruses (38), which indicates the enetically stable of ApLV populations (Table 3) .The overall lake of nucleotide diversity and high haplotype diversity within differnt sub-populations considered as a recent population expansion event.

Low genetic diversity can be caused by evolutionary founder effects/bottlenecks (39) or strong selection pressures (e.g. due to host adapta-tion).

Totally, long time attendance of viral infection in a perennial host, functional constraints during the virus life and gene flow between populations provided a favorable condition for the evolution of genetically related variants of ApLV population and bring about low-frequency polymorphism.

One of the major evolutionary factors which speed up the differentiation between homo-logous proteins is selection pressure. The dNS/dS ratio for various proteins ecncoded by ApLV (Table 3) was not different from other plant RNA viruses, and they are under negative (purifying) selection (38). The phenomenon of codon usage bias which refers to dissociation in the frequency of incidence of synonymous codons has been indicated among the broad range of organisms, including viruses (40). Always codon usage patterns indicative equilibrium exchange between the neutral selection and directional mutation pressure which contribute to translational efficiency of genes (40).

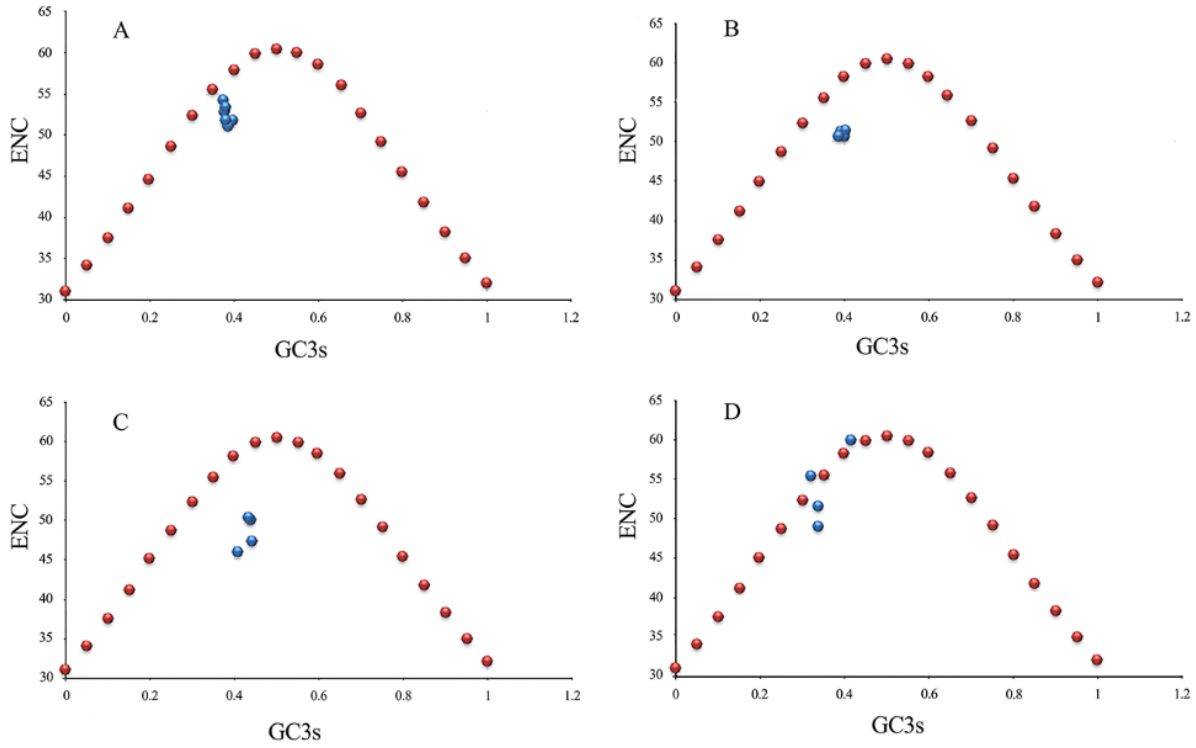

Fig. 5. ENC-plot analysis using complete gene sequences of ApLV. (A) CP gene, (B) RdRp gene, (C) TGB1 gene, and (D) TGB2 gene. The standard curve using the codon usage bias based on the GC3s composition only indicated by red points.

In this study population structuring analysis of ApLV is considered using different approches. We show the recombination breakpoints in the genome of ApLV. Furthermore, the ENC-Plot and CAI analysis are evaluated for the first time in this study to better explains the evolutionary process and fitness of ApLV.

Conclusion

In this study, nucleotide sequences of different ApLV isolates have been analyzed and our results indicated that recombination, mutation and selection pressure are the major sources of genetic differentiation among ApLV isolates.

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Conflict of interest

Both authors contributed equally as first authors. The authors have no conflicts of interest to declare that are relevant to the content of this article.

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