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Occurrence, Distribution, and Molecular Characterization of Apple Stem Pitting Virus in Iran

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ABSTRACT

Apple Stem Pitting Virus **(ASPV) is one of the most common viruses of apple and pear trees. During 2012 to 2013, a total of 1053 symptomatic and asymptomatic leaf samples were collected from orchards in nine provinces of Iran. ASPV infection was detected by DAS-ELISA and PCR in 54 samples (5.12%) from seven provinces. Based on the geographical origin, 22 representative isolates were selected for phylogenetic analysis. Twenty-two amplicons with a size of about 370 base pair (bp) comprising partial sequence of the Coat Protein (CP) coding regions of the viral genome were sequenced. Sequence data analysis, showed that the identities of 3'-terminal region of *CP* gene at both nucleotide and amino acid levels among the Iranian isolates were 95–100% and these isolates were closer to the Asian ASPV isolates than to the other isolates. Constructed phylogenetic tree by Neighbor-joining on the basis of the 3'-terminal region of *CP* gene sequences showed that the Iranian isolates were categorized into two major groups. Furthermore, phylogenetic and population genetic analysis were carried out on the basis of 3'-terminal region of *CP* gene which revealed that ASPV isolates were not geographically resolved. Also, all values in the GABranch analysis showed a ratio of substitution rates at Non-synonymous and Synonymous sites (dN/dS) below one, suggesting strong negative selection forces during *C*-terminal region of the *ASPV CP* gene history. To the best of our knowledge, this is the first report of distribution and partial genome sequence analysis of the ASPV in Iran.**

Keywords: Asian ASPV, Coat protein, Pear, Phylogenetic analysis.

INTRODUCTION

Apple Stem Pitting Virus (ASPV) is one of the major viruses of pome fruits. In spite of its worldwide distribution in apple and pear orchards (Jelkmann and Paunovic, 2011), it has only been reported from fruits exported from Iran to Russia (Buntsevich *et al.*, 2001), but has not been traced in Iranian apple orchards. ASPV alone or in combination with *Apple Chlorotic Leaf Spot Virus* (ACLSV) and *Apple Stem Grooving Virus* (ASGV) is common in many commercial cultivars of apple and pear varieties, but often remains symptomless. Symptoms including xylem pitting in the stem of Virginia Crab and epinasty together with decline of apple "Spy 227" appear only on susceptible rootstocks of *Malus prunifolia* var. ringo, *M. sieboldii*, and *M. sieboldii* var. arborescens, (Jelkmann, 1997; Jelkmann and Paunovic, 2011). Furthermore, ASPV is responsible for serious diseases such as pear vein yellows and pear necrotic spots, pear stony pit, graft incompatibilities such as top-working disease in apple and quince (*Cydonia oblonga* Mill.), quince sooty ringspots and apple ring spot in mixed infection with ACLSV (Paunovic, 1995; Jelkmann, 1997; Paunovic and Rankovic, 1998; Schwarz and

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Jelkmann, 1998; Paunovic *et al.,* 1999; Jelkmann and Paunovic, 2011). ASPV is transmitted by grafting and spread through infected propagating material. There are no known vectors able to transmit this virus (Martelli and Jelkmann, 1998).

ASPV is the type species of the genus *Foveavirus*, in the family *Betaflexiviridae* in the order *Tymovirales*. A flexuous filamentous particle about 15 nm wide and 800 nm long in sizes (Koganezawa and Yanase, 1990, Martelli *et al.,* 2007), consisting of a single stranded positive sense polyadenylated RNA genome, 9.3 kb in size. The genome is made up of five ORFs encoding respectively, the replication-related proteins (ORF 1), the putative movement proteins (ORF 2 to 4, constituting the triple block gene), and the coat protein (ORF 5).

The nucleotide sequences of the replicase (Rodoni and Constable, 2008) and Coat Protein (CP) (Nemchinov *et al.,* 1998; Klerks *et al.,* 2001) genes as well as the 3' un-translated regions (Schwarz and Jelkmann, 1998, Kundu, 2008) have high genetic variability. Nucleic acid identity among *CP* gene sequences was found to be between 70.7-93.5% (Komorowska *et al.,* 2011) and 77.0-97.0% (Yoon *et al.,* 2014). Nucleic acid diversity among *CP* gene sequence within isolates was up to 61% in some cases (Liebenberg *et al.,* 2013). However, when the nucleotide and amino acid sequences of the 660 nt 3'-CP conserved regions were compared, sequence identity values rose to 81.1-95.5% (Wu *et al.,* 2010).

Viral coat proteins are presumed to have evolved more rapidly than proteins involved in the replication and expression of virus genomes. For this reason, the coat protein genes have often been used in phylogenetic comparisons (Callaway *et al.,* 2001), and they are also used in this work.

Since no information exists on ASPV in Iran, we aimed to determine the ASPV distribution as well as its genetic diversity in apple trees in the most important apple growing regions of Iran based on the partial sequences of the *CP* genes.

MATERIALS AND METHODS

Survey for ASPV

A survey was carried out from April 2012 to June 2013, in 110 orchards located in nine geographical districts of Iran and 1,053 samples were randomly collected from apple trees (Table 1). Many of the surveyed gardens were 5-10 years old and generally included more than one variety. The most frequent varieties were: Golden Delicious, Red Delicious, Granny Smith on Maling (m-7, m-9 and m-26) Maling-Merton 111(MM111) and MM106 rootstocks, but some local varieties were also present as seed rootstocks, i.e. Golab-e Isfahan, Shafi Abadi, and Golab-e Kohanz.

Leaf sampling was performed at random, early in the morning, from different parts of canopy to minimize effects of uneven virus distribution. With the exception of symptoms of chlorosis along the veins of leaves caused by ACLSV, no symptoms of virus infection were observed in the orchards surveyed (Figure 1).

A portion of each leaf sample (0.2 g) was subjected to Double-Antibody Sandwich Enzyme-Linked Immunosorbent Assays (DAS-ELISA) with ASPV-specific antibodies (BIOREBA, Switzerland) according to the manufacturer's instruction. Absorbance values at a wavelength of 405 nm were measured in an AD340 Beckman ELISA plate reader (USA). The threshold for detection was set at two-fold higher than the mean absorbance of three replicates of negative control (Art. No. 180043). A color change indicated a positive result, whereas negative samples remained colorless. In all tests, a visual rating of ELISA plates was in agreement with the ELISA reading. A darker yellow color developed with positive samples, giving Absorbance value (A_{405})

Table 1. The incidence of *Apple stem pitting virus* in different areas and apple cultivars.

Province	No of samples collected	*Malus domestica* cvs	ASPV	Acronym
Alborz [a] (13.8%)	65	Gala	ND	RnTnAl
		Granny Smith	ND	
		Red Delicious	ND	
		Shafi Abadi	5	
		Golab-e Kohanz	4	
		Golden Delicious	ND	
Western Azerbaijan (0%)	95	Starking	ND	RnTnAz
		Sheikh Ahmad	ND	
		Red Delicious	ND	
		Gala	ND	
		Shishehei-e Tabriz	ND	
		Granny Smith	ND	
		Golden Delicious	ND	
Qazvin (6.5%)	107	Golden Delicious	ND	RnTnNa
		Golab-e Isfahan	3	
		Shafi Abadi	4	
		Gala	ND	
		Starking	ND	
Hamadan (7.1%)	84	Shafi Abadi	6	RnTnGa
		Golden Delicious	ND	
Isfahan (0%)	266	Golab-e Isfahan	ND	RnTnIs
		Sib Torsh-e Paeizeh	ND	
		Red Delicious	ND	
		Golden Delicious	ND	
		Starking	ND	
		Granny Smith	ND	
Lorestan (7%)	85	Shafi Abadi	4	TnKh
		Golden Delicious	ND	
		Golab-e Kohanz	2	
Markazi (7.2%)	110	Shafi Abadi	8	TnMa
		Golden Delicious	ND	
		Red Delicious	ND	
Mazandaran (4.8%)	145	Shafi Abadi	7	TnJa
		Golden Delicious	ND	
		Gala	ND	
		Granny Smith	ND	
		Starking	ND	
		Shafi Abadi	6	TnTe
		Golab-e Kohanz	5	
		Golden Delicious	ND	
		Red Delicious	ND	
		Granny Smith	ND	
		Starking	ND	
Total number	1053		54	
Total percentage			5.12%	

[a] The percentage of ASPV infections in each province, ND= Not Determined ZO).

Figure 1. The symptoms caused by mixed infection of *Apple chlorotic leaf spot virus* and *Apple stem pitting virus* on apple cultivar Shafi Abadi" showing chlorosis along the leaf veins.

twice or more than those given by healthy samples.

RNA Isolation, RT-PCR, Cloning, and Sequencing

Total RNA from leaf tissue of the ELISA positive samples was extracted according to Chang *et al.* (1993) with some modifications. A one-µL aliquot of total RNA was subjected to complementary DNA (cDNA) synthesis and the rest was kept at -70°C for future use. Primers specific for 3'-terminal region of the ASPV genome (GenBank accession No. D21828) that encode part of the coat protein, ASPV sense (5'-ATGTCTGGAACCTCATGCTGCAA-3', position 8,869-8,895 nt) and ASPV antisense (5'-TTGGGATCAACTTTACTAAAAAGCATAA-3', position 9,211-9,238 nt) (Menzel *et al.*, 2002) were used for detection purposes. Synthesis of cDNA was performed using 0.4 pmol reverse primer ASPV antisense and the MMLV reverse transcriptase (Promega, USA) according to the manufacturer's instructions. Total RNAs obtained from Italian ASPV infected isolate and healthy apple leaves were used as positive and negative controls, respectively. All aliquot of cDNA product was subjected to PCR using 0.4 pmol forward primer of ASPV sense and the GoTaq®Green Master Mix (Promega, USA) according to the manufacturer's instructions. Amplification cycles were as follows: denaturation step for 5 minutes at 94°C, then, 30 cycles for 1 minute at 94°C, 1 minute at 60°C and 1 minute at 72°C, and a final extension step at 72°C for 10 minute.

A total of 22 ASPV isolates were selected for sequencing and downstream analysis (Table 2). The fragments amplified by RT-PCR were ligated into the pGEM-T Easy Vector (Promega, USA) following the manufacturer's instructions and transformed into *Escherichia coli* strain M1022 electrocompetent cells using a 2510 Electroporator (Eppendorf, Hamburg, Germany) as recommended by the manufacturer. Eight white colonies resulting from transformation of each isolate were selected randomly and used as the templates in the colony PCR screening (Sambrook and Russell, 2001) using the M13 universal primers. At least two independent clones for each amplicon were sequenced in both directions by a commercial company (Eurofins MWG Operon, Germany). Finally, a total of 22 sequences of ASPV isolates (Table 2) were selected for downstream analysis.

Phylogenetic and Population Genetic Analysis

Sequence data were edited using Vector NTI advance 11.5 software (Invitrogen

Table2. List of the partial sequences of the Iranian ASPV isolates deposited in the GenBank database and the corresponding accession numbers of the origin of the viral isolates and selected ASPV isolates, obtained from other countries representing three continents, for which the full-length genome sequences or partial CDS were obtained from the GenBank database.

ASPV Isolate/Strain	Country of Origin	Host	GenBank Accession no/Protein id
Strain MT32	Poland	Apple	AF438521
Strain ST181	Poland	Apple	AF495382
Isolate CzAp36	Czech	Apple	AJ968944
PVYV PSA-H	Germany	Pear	D21828
ASPV PA66	Germany	Apple	D21829
MHczAp	Czech	Apple	DQ003336
Isolate JG1	China	Apple	EU099580
Isolate ws	China	Apple	EU314950
Isolate 38	China	Apple	FJ619187
Isolate JIASP	Poland	Apple	FJ970957
Isolate ASPVPLM1	India	Pear	FN430678
Isolate pv6	China	Apple	HM125160
Isolate LV-m198	Latvia	Apple	HQ661836
Isolate LV-m205	Latvia	Apple	HQ661837
Isolate YN-MRS	China	Pear	JX673789
Isolate HN-ShM	China	Pear	JX673802
Isolate HN-BL	China	Pear	JX673826
ASPV-SJY-HR-YC1	South Korea	Apple	KC791784
Isolate ASPV-KJH-HR-JS3	South Korea	Apple	KC791788
Isolate PB66	United Kingdom	Apple	KF321966
R1T1Na	Iran	Apple	KM243885
R1T3AL	Iran	Apple	KM243886
R1T4Na	Iran	Apple	KM243887
R1T7Ga	Iran	Apple	KM243888
R2T6Na	Iran	Apple	KM243889
R2T7Ga	Iran	Apple	KM243890
R4T6Ga	Iran	Apple	KM243891
R14Ten-1Al	Iran	Apple	KM243892
R-1T7Na	Iran	Apple	KM243893
T1Ma	Iran	Apple	KM243894
T1Te	Iran	Apple	KM243895
T3Te	Iran	Apple	KM243896
T4Ma	Iran	Apple	KM243897
T4R4Ma	Iran	Apple	KM243898
T5Kh	Iran	Apple	KM243899
T5Te	Iran	Apple	KM243900
T6Kh	Iran	Apple	KM243901
T6Ma	Iran	Apple	KM243902
T7Ja2	Iran	Apple	KM243903
T8Kh	Iran	Apple	KM243904
T11Ja	Iran	Apple	KM243905
T12Ja	Iran	Apple	KM243906
Peach chlorotic mottle virus (Isolate Agua-4N6)	Canada	Peach	NC_009892

Corp., Carlsbad, CA, serial no. 2-2435-2555-B0F0A). The Blast-n algorithm, available through the NCBI web server (http://www.ncbi.nlm.nih.gov), was used to identify the closest matches (relatives) to putative ASPV sequences amplified and sequenced from samples collected in the present study. Based on the Blast-n results, 20 full-length/partial CDS ASPV isolates/strains were identified that collectively had matches to isolates previously described from two continents: Asia (India, China, South Korea) and Europe (Czech Republic, Germany, Latvia, Poland, UK) (Tables 2). *Peach chlorotic mottle virus* was used as an outgroup in the analysis. Viral nucleotide and amino acid partial sequences were aligned using CLUSTAL W, implemented in MEGA6 software (Tamura *et al.*, 2013), with the default parameters. The sequences were subjected to identity matrix estimation and recombination analysis, phylogenetic reconstruction, and genetic variability analysis.

BioEdit version7.1.3.0 (Hall, 1999) was used to determine identities based on deduced amino acid (aa). Pairwise distances were calculated using the PASC algorithm available at the GenBank database (http://www. ncbi.nlm.nih.gov/sutils/pasc/) (Bao *et al.*, 2012).

The codon-based genetic algorithm GABranch method was used to evaluate if the studied isolates had evolved due to positive selection. This method can partition automatically all branches of the phylogeny describing non-recombinant data into groups according to the ratio of *dN/dS* (the average number of non-synonymous (amino acid altering) substitutions per Non-synonymous sites (dN) to the average number of synonymous (silent) substitutions per Synonymous sites (dS)).

The most appropriate model was determined using the Bayesian Information Criterion (BIC) implemented in MEGA6. Using MEGA6, a Neighbor-Joining (NJ) tree was reconstructed, with the evolutionary model Kimura 2-parameter +G and 1,000 bootstrap iterations (Kimura, 1980) at nucleotide level and a Maximum-Likelihood (ML) tree was constructed based on the Le Gascuel 2008 model (Le and Gascuel, 1993) with 1,000 bootstrap iterations at deduced amino acid level.

RESULTS

ASPV Detection and Distribution

All of the 1,053 tested apple trees were individually inspected. Out of the 1,053 collected leaf samples tested by DAS-ELISA, ASPV was detected in 54 samples (5.12%) including *Malus domestica* cv., and cv. from seven provinces including Alborz, Hamedan, Lorestan, Qazvin, Markazi, Mazandaran and Tehran (Table 1). The infection rate of ACLSV was particularly significant in the local varieties Shafi Abadi, Golab-e Kohanz and Golab-e Isfahan (100%). Among the apple trees infected with ASPV, *cv.* Shafi Abadi, a seed rootstock, was found to have resulted from mixed infections with ACLSV and showed symptoms of chlorosis along the veins of leaves (Figure 2), but other apple cultivars were symptomless (Table 1). Our results showed that ASPV was widely distributed in the northern and central regions of Iran. Rates of infected trees varied from no infection in Western Azarbaijan, and Isfahan Provinces to 13.8% in Alborz Province.

A PCR-amplified product of approximately 370 bp, corresponding to the 3'-terminal region of the *CP* gene of ASPV, was obtained for all the ELISA positive samples, while, the expected DNA was not amplified in 10 ELISA negative samples collected from Western Azarbaijan, and Isfahan Provinces. All sequences obtained in the present work, without primers regions, were deposited in GenBank under the assigned accession numbers KM243885 to KM243906 (Table 2). Based on pairwise comparisons using PASC (Bao *et al.*, 2012) and sequence identity matrix using BioEdit (Hall, 1999), all of the Iranian ASPV

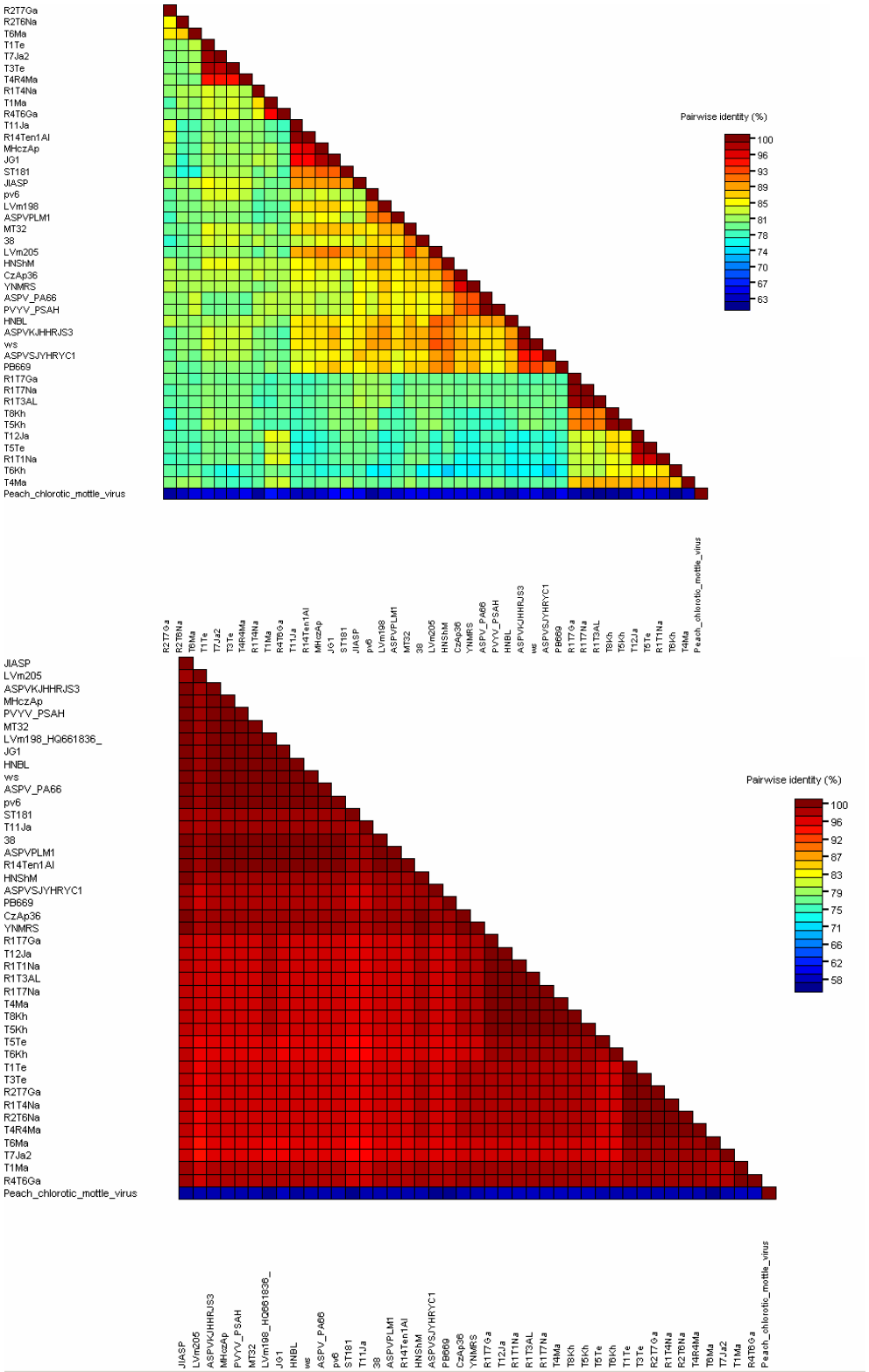

Figure 2. 2D Graphical representation of pairwise nucleotide (left diagonal) and deduced amino acid (right diagonal) comparisons with the percent identity scale in the right side.

isolates were found to share > 95% amino acid (aa) sequence identity (95.6-100%) with other ASPV isolates in the *C*-terminal region of the viral *CP* gene.

Genetic Diversity and Selection Pressure Analysis

Sequence analysis data revealed that the 3' end of CPs sequenced consist of 311-318 nt in size. The identities of the 3' end of CP at nucleotide and amino acid levels among all 22 Iranian isolates were 76.4-99.60% and 94.5–100%, respectively, of which the T11Ja (KM243905) and R14Ten-1Al (KM243892) isolates were the most distant ones (Figures 2 and 3). Comparisons of the Iranian isolates with the available ASPV sequences in GenBank based on *aa*

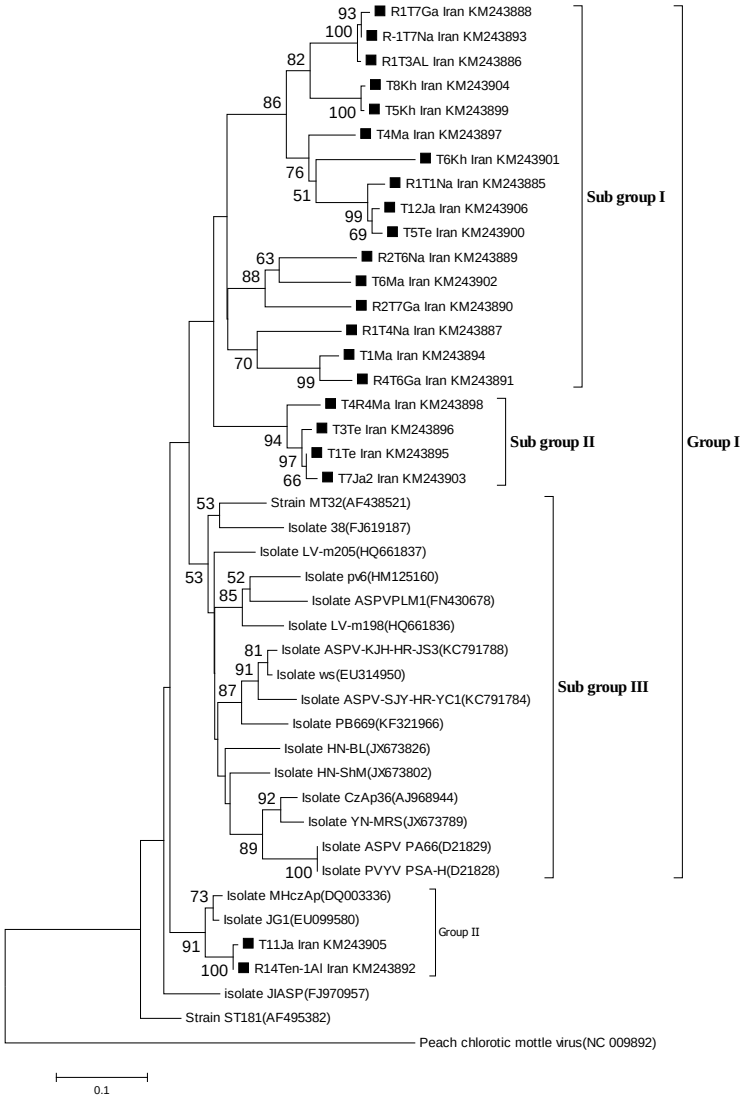

Figure 3. Phylogenetic tree of the partial nucleotide sequences of isolates (n= 42) of ASPV strains/isolates from other regions of the world, reconstructed using the Neighbor-Joining (NJ) method based on the Kimura 2-parameter method (Kimura, 1980) and the rate variation among sites as modeled with a gamma distribution. The NJ tree is rooted using *Peach chlorotic mottle virus*, and the percentage bootstrap support values (1,000 iterations) are indicated at the major nodes. Bootstrap values > 50 are indicated. The scale bar shows the number of nucleotide substitutions per site. Iranian isolates are marked with black square.

sequences, showed that the R14Ten-1Al and T11Ja isolates were closely related to the MHczAP isolate from Czech Republic (DQ003336) with 100 and 98.9% identity, respectively. The isolates T1Ma, T6Ma, T4R4Ma, T4Ma, T8Kh, T5Kh, T6Kh, T7Ja2, and T12Ja were the closest isolates to the Chinese isolate HN-ShM (JX673802) (> 97.8% identity); and R1T7Ga, R2T7Ga, T1Te,T3Te, R1T1Na, R-1T7Na, R1T4Na and R1T3Al isolates were the closest isolates to the other Chinese isolate YN-MRS (JX673789) with 98.9% identity (Figure 3), suggesting that Iranian ASPV isolates are more closely related to Asian than European isolates. In all cases, the identities among the isolates based on *aa* sequences were in accordance with those of nt sequences.

The genetic diversity of all 22 Iranian ASPV isolates was studied by sequencing of the CP region (8,869-9,238; 370 bp) and aligning a matrix of 276 bp with data of other ASPV isolates from GenBank. The analysis also included another member of *Foveavirus*: *Peach chlorotic mottle virus* (NC-009892) which was used to root the tree.

When nucleotide sequences were considered, the ASPV isolates were divided into two major groups (Figure 3). Group I is divided in three subgroups and the majority of the Iranian isolates were scattered in both subgroups I and II, but not in subgroup III, which comprised isolates from China, Czech, Grmany, India, Lativa, Poland, South Korea and Uk.

Interestingly, within Iranian isolates, only T11Ja and R14Ten-1Al clustered in the Group II together with isolates MHczAp (Czech) and JG1 (China). The two Polish isolates (JIASP and ST181) remained ungrouped.

In the tree reconstructed on the basis of the *aa* sequences of the partial sequence of CP, only the Iranian ASPV isolates formed a common cluster but the other ASPV isolates remained ungrouped (Figure 4). Confirming results were obtained from nucleotides sequence analysis, the two Iranian isolates T11Ja and R14Ten-1Al were not grouped into the latter cluster.

Also, to determine whether the Iranian ASPV isolates evolved due to positive selection, the 3' end of CP of the 22 Iranian ASPV isolates (Table 2) were aligned together and analyzed using GABranch. All values in this GABranch analysis showed dN/dS ratios below one, also indicative of negative selection during the history of the *C*-terminal region of the *ASPV CP* gene on the Iranian isolates considered (Figure 5).

Collectively, these results serve as indicators of high genetic variability among the Iranian ASPV isolates when compared to the ASPV isolates from GenBank (Table 4). Despite the high variability of the *CP* gene sequences, all values in this GABranch analysis showed omega values below one. Thus, high levels of variability in the *CP* gene sequences of our isolates resulted from a high rate of synonymous mutations.

DISCUSSION

Our results showed that the incidence of ASPV in the major apple production areas of Iran was 5.1%. This incidence can be considered low when compared with those of other countries such as Greece where the incidence of ASPV in apple and pear orchards was found to be 91.8 and 51.3%, respectively (Gadiou *et al.,* 2010). The extraordinary outbreak of ASPV in this country could be due to the low sanitary status of apple and pear trees in Greece (Mathioudakis *et al.*, 2010). The low ASPV distribution is in line with previous study on pome fruit trees, reported from Egypt (Youssef *et al.,* 2009). In fact, as ASPV and ACLSV are frequently latent (Jelkmann, 1997) in appropriate surveillance of propagation plant material favors their dissemination. Also, the common practice of grafting is an effective way to transmit viruses and may cause their wide distribution. The absence of ASPV infection in the Iranian provinces of Isfahan and West Azarbaijan could be due to the absence of

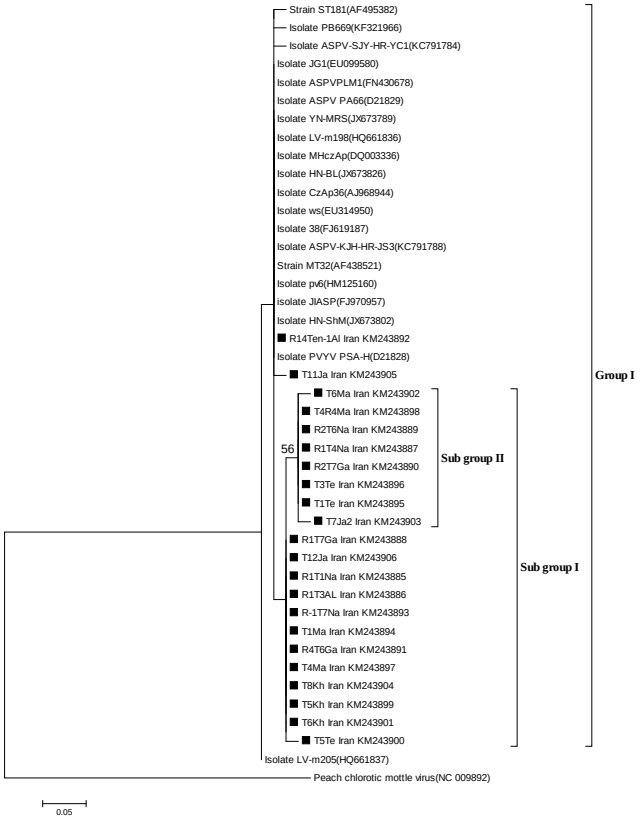

Figure 4. Phylogenetic tree of the partial amino acid sequences of isolates (n = 42) of ASPV strains/isolates from the other regions of the world, reconstructed using the Maximum Likelihood method based on the Le _ Gascuel_2008 (LG) model (Le and Gascuel, 1993). The tree has rooted using *Peach chlorotic mottle virus* and the percentage bootstrap support values (1,000 iterations) are indicated at the major nodes. Bootstrap values > 50 are indicated. The scale bar has shown the number of nucleotide substitutions per site. Iranian isolates are marked with Black Square.

the viral inoculum in these regions. National Plant Protection Organization of Iran prevents the import of virus infected material into the country. The national certification program for propagating material of pome fruits was assessed during the 2008 (Article is available at http://www.spcri.ir/). Perhaps the absence of an efficient certification program of plant propagating materials has favored the occurrence and spread of this virus.

Sequence comparisons revealed that the 22 Iranian ASPV isolates shared considerable identity with each other at both nt and *aa* levels (95–100%) and indicated that they were closer to some of the Asian isolates of ASPV (Figure 3). High nucleotide and amino acid identity in the 3'- terminus of the *CP* gene was also observed in European and Asian population of the virus (Wu *et al.*, 2010) suggesting that ASPV has a conserved *C* terminal of the *CP* gene. As a general assumption, RNA viruses are more genetically variable than the others, because of the lack of proofreading activity associated with RNA-dependent RNA polymerase so that single host may contain co-existing populations of diverse sequence variants (Yoshikawa *et al.*, 2001). In the case of ASPV, the complete nucleotide sequences of *CP* gene have been determined and sequence analysis has shown high variability (Komorowska *et al.*, 2011). In particular, two variable regions of the ASPV

genome are located between the MET and P-Pro domains in the replication protein ORF1 (Yoshikawa *et al.*, 2001) and in the *N*-terminal CP coding region, whereas the *C*-terminal region is conserved (Yoshikawa and Takahashi, 1988; Nemchinov and Hadidi 1998; Yoshikawa *et al.*, 2001; Yoon *et al.*, 2014). On the basis of the 3'-terminal region of *CP* gene sequences, the Iranian isolates studied were categorized into two major groups. The phylogenetic analysis based on this partial sequence showed that ASPV isolates were not discriminated based on their geographical regions (Figures 3 and 4) and did not allow to draw conclusions on their origin and dispersion, which is inconsistent with the previous report from Gadiou *et al.* (2010) who studied genetic diversity of 13 ASPV isolates by sequencing the CP region (8,322-9,133 bp) and aligning a matrix of 802 bp with data of other ASPV isolates from GenBank. One probable reason is that the *C*-terminal region of *CP* gene is conserved which is in accordance with the previous report (Yoon *et al.*, 2014).

The overall *dN/dS* ratio for the 3'-terminal region of the *CP* gene was less than 1.00 indicating negative or purifying selection which has probably acted to preserve the encoded protein function and their importance in virus life cycle. Therefore, it could be concluded that the 3'-terminal region of the *CP* gene is under a high selection pressure (dN/dS= 0.0150) and more conserved than the 5'-terminal regions of the *CP* gene (Figure 5). Low values of *dN/dS* have been reported in different genomic regions for the other members of the family *Betaflexiviridae* (Chare and Holmes, 2006; Teycheney *et al.*, 2005; Komorowska *et al.*, 2011; Yoon *et al.*, 2014) indicating that the attribute is common to the members of this family.

Future studies are needed to evaluate the genetic diversity of Iranian ASPV isolates based on the full length sequences of the viral genome. However, even if commercial apple tree varieties remain symptomless after infection, growth and crop yield can be reduced (Salmon *et al.*, 2002). For this

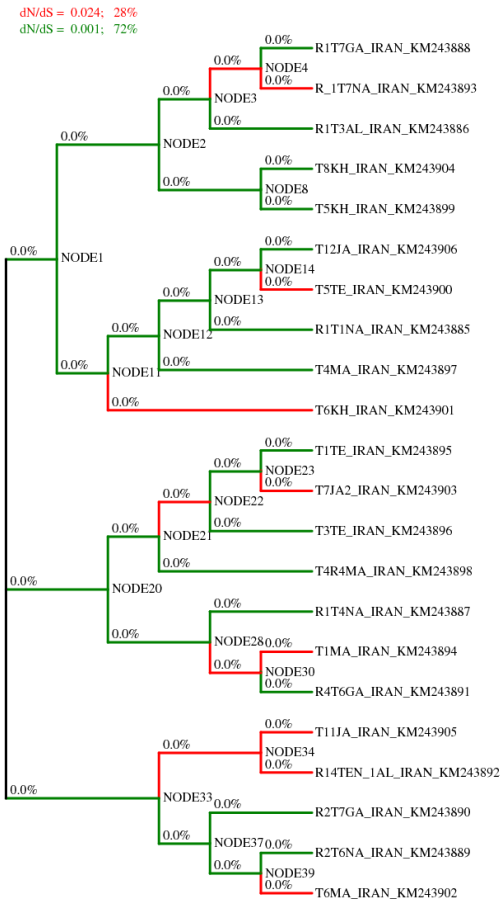

Figure 5. GABranch analysis showing a Neighbor-Joining tree with levels of omega coded by colors. Green branches represent the highest *dN/dS* values, and red colored branches indicate medium values. All the values in this GABranch analysis show omega below 1 suggesting strong purifying selection forces acted during the analyzed ASPV CP history.

reason, the first incidence of infection emphasizes the need for certification schemes for the production of virus-free propagating material and it warrants a comprehensive study on its sources and its mode of spread and survival in this country.

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وقوع، پراکنش و ویژگی‌های مولکولی ویروس ساقه گودکی سیب درایران

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چکیده

ویروس ساقه گودکی سیب (ASPV) یکی از رایج‌ترین ویروس‌های درختان سیب و گلابی است. در مجموع ۱۰۵۳ نمونه برگی طی دو سال زراعی ۱۳۹۱ و ۱۳۹۲ از نه استان بصورت تصادفی جمع آوری شد. آلودگی به ASPV با استفاده از آزمون الیزای مستقیم و پی‌سی‌آر در ۵۴ (۵/۱۲٪) نمونه از هفت استان ردیابی شد. براساس مناطق جغرافیایی، ۲۲ جدایه به منظور تجزیه و تحلیل‌های فیلوژنتیکی انتخاب شد. بیست و دو قطعه به اندازه ۳۷۰ جفت باز مربوط به قسمتی از ژن رمز کننده پروتئین پوششی تعیین ترادف شدند. تجزیه و تحلیل توالی‌ها نشان داد که جدایه‌های ایرانیASPV در هر دو سطح نوکلئوتید و آمینواسید (۹۵-۱۰۰٪) با یکدیگر برابری قابل توجهی دارند و به دیگر جدایه‌های آسیایی ASPV نزدیک هستند. درخت فیلوژنتیکی با روش نزدیکترین همسایه، Neighbour-joining (NJ) ترسیم شده بر اساس انتهای آمینی ژن پروتئین پوششی نشان داد که جدایه‌های ایرانی ASPV به دو گروه بزرگ تقسیم شدند. این دسته‌بندی را با تفاوت در منشاء جغرافیایی جدایه‌ها نمی‌توان تفسیر کرد. همچنین براساس داده‌های GABranch، برآورد نسبت dN/dS در انتهای کربوکسیل پروتئین پوششی ASPV کمتر از یک بود، که مؤید فشار انتخاب منفی یا پالایشی روی این ناحیه است. براساس اطلاعات موجود، گزارش حاضر اولین گزارش ازتعیین پراکنش و تعیین ترادف قسمت هایی از توالی ژنوم جدایه های ASPV در ایران است.