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Original Article

Phylogenetic analysis of Iranian Apple Stem Grooving Virus Isolates Using Coat Protein Gene

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Abstract

Background and Aims: Apple stem grooving virus (ASGV) is one of the economically important latent viruses infecting pome fruit trees worldwide. The investigation on the distribution of ASGV and its molecular properties can give us more information about the epidemiology and control of this latent and graft-transmissible virus.

Materials and Methods: The presence of ASGV in apple and pear orchards in Mazandaran province (Northern Iran) was investigated using serological and molecular approaches. The phylogenetic tree was constructed by the Neighbor-Joining method implemented in MEGAX. Two-dimensional nucleotide diversity plot constructed using CP sequences by SDTv1.2 version 12. RDP4 and Dnasp software were used for recombination, and genetic diversity and demographic analysis, respectively v. 6.10.04.

Results: ASGV was detected in 10.2% of leaf samples by ELISA test. A DNA amplicon with the expected size of 780 bp corresponding to the ASGV coat protein (CP) gene was amplified using RT-PCR assay. Phylogenetic analysis showed that the ASGV isolates clustered into two main groups, which is confirmed by the nucleotide identity plot. Group II (GII) includes two Chinese and Korean pear isolates with an apple isolate from India. However, most of the ASGV isolated from different geographical regions including Iranian isolates fell into group I (GI). High nucleotide identity (91 to 100%), low nucleotide, and high haplotype diversities indicate the recent distribution of ASGV. No clustering was found according to the hosts or where the ASGV was isolated. Using dN/dS values it was found that the different populations of ASGV are under negative (purifying) selection with the ω ratio less than 1. The highest gene flow was determined between American and South Asian populations. Moderate or low genetic differentiation, and frequent gene flow (FST < 0.33 and Nm > 1) also confirmed with Ks*, Kst* Z, Z* and Snn statistics values, except between West Asia and East Asia populations which may suggesting these isolates are older than the others.

Conclusion: To our knowledge, this is the first report of ASGV infecting apple and pear trees in North Iran, which extends the geographical range of this viral disease. Accordingly, larger-scale ASGV investigations must be conducted to determine the distribution and prevalence of this virus in Iran.

Keywords: ASGV, Mazandaran, DAS-ELISA, RT-PCR, Phylogenetic analysis

Introduction

Apple stem grooving virus (ASGV), is the type species of genus Capilovirus in the family Betaflexiviridae (1). Since the first report of ASGV in the 1960s from the USA (2), now it is distributed worldwide and has been identified in many horticultural crops (1). The main hosts of this virus include species in Liliacea, Actinidiaceae, Rutaceae, and Rosaceae families. ASGV is one of the most important viruses infecting apple globally and can cause severe economic losses (3, 4).

Most commercial apple and pear cultivars are symptomless unless they are grafted onto a

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susceptible rootstock (5). Some different symptoms of ASGV infection include skin swelling, chlorotic leaf spots, brown lines in the upper part of the graft, leaf chlorosis, holes or ring spots, and stem grooving in the stem (5, 6). Since ASGV attacks the wood tissue, the fruits become small, and trees may dry up. The infection of ASGV in loquat (Eriobotrya japonica), kiwifruit (Actinidia chinensis), lily (Lilium spp.), fig (Ficus palmata), Nandina domestica, lotus (Nelumbo nucifera), and Japanese apricot (Prunus mume) has also been reported (7-12). ASGV is easily transmitted mechanically, but no natural vector is reported (13). In some hosts like Malus platycarpa, L. longiflorum, and Chenopodium quinoa seed transmission has been reported (6).

ASGV has flexuous filamentous virions, which contain single-stranded (ss) positive sense monopartite RNA with capped 5´ and a polyadenylated 3´ ends. The genome of ASGV is about 6.5 kb, consisting of two open reading frames (ORFs) (1). ORF1 encodes replicase (Rep) and coat protein (CP) products. ORF2 is nested within ORF1 and encodes a movement protein (MP). Previous research found that inserting a stop codon between Rep and CP coding regions of ASGV maintained systemic infection in plants, indicating a subgenomic RNA (sgRNA) strategy for CP expression (14). Phylogenetic analysis using complete gene sequences of ASGV isolates clustered them into four groups. However, based on the CP gene three groups were identified (15).

Together molecular analysis of ASGV from various sources demonstrated no manifest correlation between molecular diversity and geographical regions or hosts (16, 17).

Considering the importance of the viral diseases, and damages in pome fruits, identifying viruses and investigating their genetic variation is very important for improving disease management programs. The ASGV infection was first reported in Iran from apple and pear gardens in West Azerbaijan and Khorasan Razavi provinces (18). In addition, molecular analysis of Iranian ASGV isolates using the CP gene from ASGV isolated from apple and citrus trees in the Northwest and West of Iran was investigated (19). Due to the importance and economic losses of ASGV, in this research, we focused on the serological and molecular detection of this virus from apple and pear orchards in Mazandaran province (North Iran). Our analysis indicates the ASGV infection of apples in North Iran, which extends the geographical range of this viral disease. Accordingly, larger-scale ASGV investigations must be conducted to determine the distribution and prevalence of this virus in Iran.

Methods and Methods

Sample Collection

A survey of ASGV occurrence was conducted from 2019 to 2020 in 17 orchards located in Kiasar, and Nowsar Mazandaran, Iran. A total of 107 leaf samples, including 102 apples, and five pear leaf samples were collected (Table 1). As ASGV is a symptomless virus in some apple cultivars, symptomatic and symptomless trees were sampled. Symptomatic samples showed leaf vein clearing, leaf deformation, leaf chlorosis, growth reduction, and decline. Leaf samples were collected from different parts of the tree canopy to minimize the effects of non-uniform virus distribution, and all samples from each tree were pooled and used as one sample.

ELISA. The collected samples were screened by DAS-ELISA (20), using ASGV-specific polyclonal antibodies (Bioreba, Switzerland) according to the manufacturer's recommended protocol. The specific antibody was used to coat microtiter plates (NUNC, Denmark) at a 1:1000 dilution, and incubated at 4ºC over-night. Homogenized sap (100 µl per well) was added to duplicate wells of the plate and incubated at 4ºC for one night. Specific ASGV conjugated antibody was added (100 µl per well), and incubated for 4 hours at 37ºC.

Alkaline phosphatase substrate (Sigma Chemical Co.) was used, and absorbance values were measured at 405 nm using BioTek (USA) microplate reader. A sample was considered positive if the absorbance means of negative control plus three times the standard deviation of the negative control.

Extraction of RNA. Total RNA was extracted from eleven ELISA-positive leaf samples using

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the modified CTAB method described by Chang et al. (21). The leaf samples (0.2 g) were ground in liquid nitrogen, and 600 µl of the extraction buffer (100 mM Tris-HCl, 2% CTAB, 2% PVP, 2M NaCl, 1% sodium sulfite, 20mM EDTA) was added to micro-tube. After incubation at 65 °C for 5 min, an equal volume of chloroform-isoamyl alcohol (24:1) was added, roughly shacked, and centrifuged at max speed (15000g) for 15 min (at room temperature). An equal volume of lithium chloride (5 M) was added to the clear upper phase gently mixed, and incubated at 4ºC overnight. The pellet was resuspended in 200 µl of TE buffer containing 1 % SDS, gently mixed with 100 µl of 5M NaCl and 300 µl of cold isopropanol. Total RNA quality was checked by electrophoresis in agarose gel containing ethidium bromide.

RT-PCR. All eleven ELISA-positive 11 ELISA samples were checked by RT-PCR using specific primers designed to amplify ASGV CP gene (20). One microliter of reverse primer (20 pmol/µl) ASGV-R (5'-GCGACCA-AGTTTGCGGAATTTCACA-3') (22), and 4 µl of total RNA were added to 16 µl of the reverse transcription (RT) mix (2 µl of 5X RT buffer, 1 µl of dNTPs (10 mM), 0.5 µl of RNase inhibitor enzyme (200 U/µl). Then incubated at 65°C, for 5 min, and immediately chilled on ice. M-MuLV reverse transcriptase (Fermentas-Lithuania) was added, and the reactions were incubated at 42°C, for 1 hour. The PCR reaction was done in final volume 25 µl using ASGV-F: 5-'GTCCCTCTCGGCTA-GAATTGAAAGAT-3', and reverse prime (20 pmol/µl). PCR program was 94°C for 5 min, 35 cycles with 94°C for 1 min, 61°C for 1 min, and 72°C for 1 min, and the final extension step at 72°C for 10 min. The amplified PCR products were analyzed on 1% agarose gel. Two DNA amplicons of apple isolates were chosen based on their different collection regions (Fig. 2) and their nucleotide sequences were determined using the Sanger method in both directions by Macrogen Inc (Seoul, South Korea) service.

Sequencing and Phylogenetic Analysis. The obtained two complete CP sequences from apple trees (Nowsar and Kiyasar counties) were compared with the sequences of other ASGV isolates available in the GenBank by BLASTn program (http://www.ncbi.nlm.nih.gov). Multiple sequence alignments were done using the Muscle, implemented in MEGAX software, and the alignments were used for further analysis .

Phylogenetic tree reconstructed by the Neighbor-Joining (NJ) method with 1000 bootstrap replicates by MEGAX. Cherry virus A (Acc. No. NC_003689) was used as an out-group (22). The overall mean diversity between ASGV CP gene sequences was estimated using Kimura two-parameter method implemented in MEGAX (23). Two-dimensional nucleotide diversity plot constructed by CP sequences using SDTv1.2 version 12.

Recombination Analysis.

RDP4 (http://web.cbio.uct.ac.za/~darren/rdp.html) (24) was used to determine the presence of recombinant nucleotide sequences. Within RDP4, six different methods were used to assess the sequences having recombination breakpoints: RDP, BootScan, GENECONV, MaxChi, and SiScan. Default RDP4 settings were used throughout and sequences only with the breakpoints having Bonferroni-corrected p-value ≤ 0.05 were considered true recombinants.

Genetic Diversity and Demographic Analysis.

For genetic diversity and demographic analysis 337 CP nt sequences were obtained from GenBank and clustered according to geographical isolation including AM (America; n=17), EA (East Asia; n=222), SA (South Asia; n=87), and WE (West Asia; n=11). The DnaSP v. 6.10.04 (25) is used for population genetic parameters according to geographical distribution and original host. Analyses of nucleotide and haplotype diversity were conducted separately for each population as well as for all populations grouped together.

The confidence intervals of the number of haplotypes (h), haplotype diversity (Hd), average number of nt differences between the sequences (k), nt diversity (per site) (Pi), total number of synonymous sites (SS), total number of non-synonymous sites (NS), and the ratio of non-synonymous to synonymous nt diversity ($\omega = dN/dS$), on the CP nt sequences

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was calculated (25). The gene is under positive (diversifying), neutral, and negative (purifying) selection when the ω ratio is > 1, = 1, and < 1, respectively (25). Tajima's D, and Fu and Li's F* statistical tests (without outgroup, window length: 100 sites and step size: 25 sites) were performed using DnaSP (25).

Neutral Selection Analysis. The differences between the number of segregating sites and the average number of nucleotide differences became the basis for Tajima's D test (26). The differences between the number of singletons and the average number of nucleotide differences between the pairs of sequences are the basis for Fu and Li's F* test (27).

Gene Flow and Genetic Differentiation among Populations. A The Ks *, Kst*, Z*, Snn and Fst values (28) which determine the genetic differentiation on the CP nt sequences were calculated using DnaSP (25). Kst* will be near zero if there is no genetic differentiation (null hypothesis). A smaller Z* means a smaller genetic differentiation among the population (29). The value of Snn describes a range of the exact same population (value of 0.5) (null hypothesis) to distinctly differentiate the population (value of 1) (28).

The null hypothesis in Ks *, Kst*, Z*, and Snn is rejected by a significant P value. Fst ranges between the exact same population (value of 0.0) to fully distinct populations (value of 1.0) (28). Fst> 0.33, in most cases, indicates infrequent gene flow and a big genetic differentiation in the tested populations (30). The levels of gene flow can be categorized as Nm > 1 (high gene flow), 0.25 to 0.99 (intermediate gene flow), and Nm < 0.25 (low gene flow) (31).

Results

Most of the apple leaf samples were asymptomatic, however, some symptomatic samples with leaf vein clearing, leaf deforma-tion, leaf chlorosis, and growth reduction were collected. The result of the serological assay is shown in Table 1. Based on DAS-ELISA 10.2% of 107 leaf samples (asymptomatic and symptomatic) were found to be infected by ASGV. The highest ASGV infection was found in asymptomatic apple and symptomatic pear leaf samples in Amole (17.39%), and Sari (50%), respectively. Among the pear samples only two symptomatic leaf samples from Kiyasar (Fig. 1) showed positive reaction in ELISA test (Table 1)

Fig. 1. Leaf deformation in pear isolate (PNE2) infected with Apple stem grooving virus.

RT-PCR and Sequencing

A RT-PCR on ELISA-positive samples using specific primers resulted in the amplification of a DNA fragment with the expected size of 780 bp (Fig. 2). RT-PCR results and sequencing data confirmed the presence of ASGV in eleven ELISA-positive samples.

The nucleotide sequences of the CP gene of two ASGV isolates from apple trees in Nowsar and Kiasar, were determined. A BLAST search of the obtained sequences with other sequences in NCBI confirmed the ASGV presence. The both sequences were 714 nucleotides long, covering the full length of the CP gene coding sequence. The sequences were deposited in the GenBank database, with acc. Nos. MZ773528, and MZ773529 for Kiyasar and Nowsar ASGV isolates, respectively.

The phylogenetic tree reconstructed using 93 ASGV CP gene sequences showed two main groups, which were supported by bootstrap analyses (Fig. 3). Although most of the ASGV

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isolates from different geographical regions and hosts, including Iranian isolates fell, into GI group, two pear isolates, one from China (KR185346) and the other from South Korea (LC475148), with an apple isolate from China (MK481973), were placed in the other group (GII) (Fig. 3). No manifest correlation between molecular diversity and geographical regions or hosts was inferred from the phylogenetic tree. A two-dimensional nucleotide identity plot constructed by SDTv1 showed that ASGV isolates were placed in two main groups, in accordance with the phylogenetic tree (Fig. 4). The lowest and highest nucleotide identities were 91% to 95% and 96% to 100% for ASGV isolates in GII and GI groups, respectively.

The overall mean nucleotide divergence and haplotype diversity among the ASGV CP gene were 0.027 ± 0.002, and 0.9955, respectively. In addition, the overall mean nucleotide diversity for Iranian ASGV isolates was 0.011± 0.003, with more than 99% nucleotide identity.

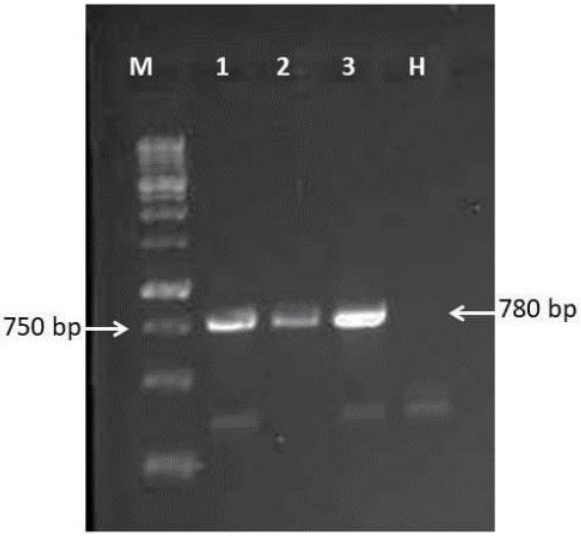

Fig. 2. RT-PCR detection of *Apple stem grooving virus* using specific *CP* gene primers. M: Molecular marker (1kb DNA ladder, Fermentas); Lane 1: the apple isolate (Nowsar, acc. No. MZ773529); Lane 2: pear isolate (PNE2); Lane 3: the apple isolate (Nauni, acc. No. MZ773528). Lane 4: Healthy plant.

Table 1. Table 1 Detection of positive samples by DAS-ELISA

Location (counties)	Hosts	No. of tested samples		No. of positive samples	positive samples (%)		No. of sequenced samples
		asymptomatic	symptomatic		asymptomatic	symptomatic	
Kiyasar (Sari)	Apple	40	29	4	7.5	3.45	1
	Pear	0	4	2	0	50	0
Nowsar (Amol)	Apple	23	30	5	17.39	3.33	1
	Pear	0	1	0	0	0	0

Table 2 Summary of the demography test statistics between the ASGV *CP* gene populations

population	N	h	Hd	K	Pi	dS	dN	ω	Neutrality test and significance test	
									Tajima's D	Fu's Lis F
All	337	283	0.998	55.641	0.08029	0.288 ±0.026	0.018 ±0.002	0.062	-1.31239	-225.594
AM	17	15	0.985	48.735	0.06826	0.246 ±0.027	0.013 ±0.003	0.052	0.3292	0.376
EA	222	198	0.998	55.385	0.07992	0.281 ±0.023	0.019 ±0.003	0.067	-1.15273	-134.355
WE	11	10	0.981	17.854	0.02501	0.092 ±0.013	0.003 ±0.001	0.032	-1.76742 *	-0.845
SE	87	60	0.986	45.508	0.06374	0.226 ±0.024	0.017 ±0.002	0.075	-0.66859	-4.965

N – number of isolates; h – number of haplotypes; Hd – haplotype diversity; k – average number of nucleotide differences between sequences; Pi– nucleotide diversity (per site); dS – synonymous nucleotide diversity; dN – non-synonymous nucleotide diversity; ω – dN/dS; *P < 0.05; ns – not significant;

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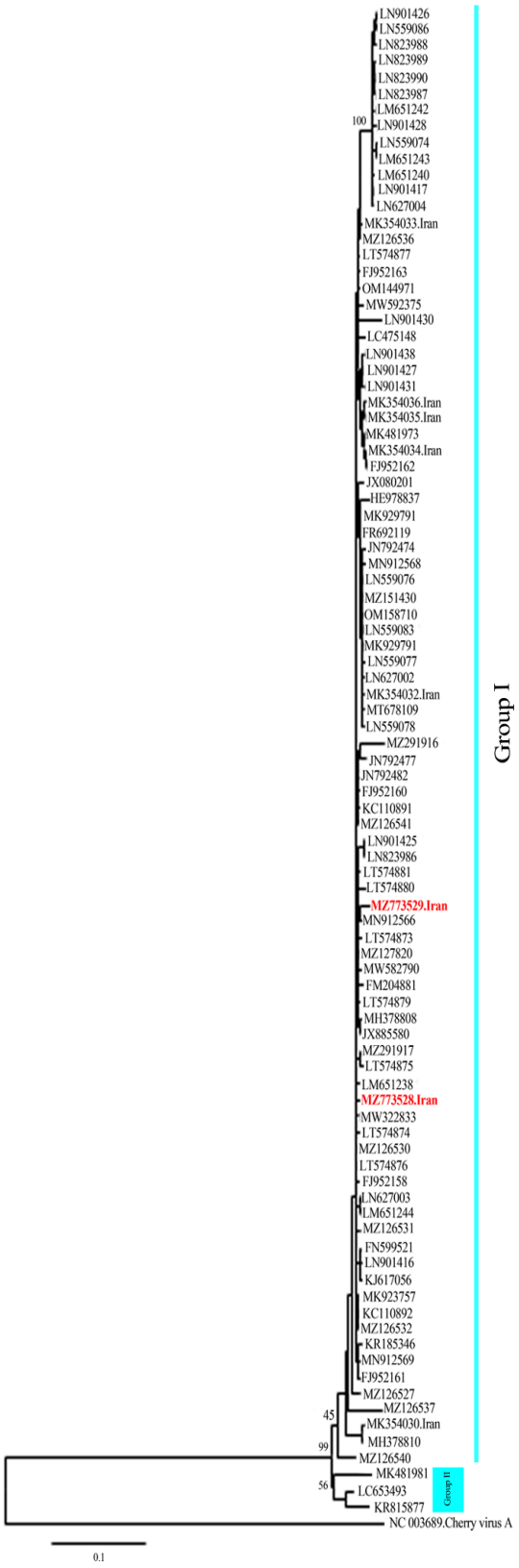

Fig. 3. Phylogenetic tree constructed by Neighbor-joining method using CP nucleotide sequences of *Apple stem grooving virus* isolates available at GenBank including Iranian isolates**.** *Cherry virus A* (NC_003689)

Fig. 4. Two-dimensional nucleotide identity plot constructed by *Apple stem grooving virus* CP sequences available at GenBank including Iranian isolates.

Genetic Diversity and Polymorphism Analysis

In the *CP* gene analysis, 283 distinct haplotypes were detected among the 337 tested isolates. Haplotype diversity (Hd) for all 337 sequences was calculated to be 0.98690. The average number of nucleotide differences (k) was shown to be 55.64155, and nucleotide diversity (Pi) was 0.08029 (Table 2).

The highest haplotype diversity (Hd), and nucleotide diversity (Pi) were detected in East Asia population (Table 2).

However, Nucleotide diversity (Pi) and haplotype diversity (Hd) are lower in West Asia (Table 2). Nucleotide differences within haplotypes (k) are highest in East Asia (55.38539), followed by American, South Asian, and West Asian populations. Using dN/dS values, it was found that the different populations of ASGV are under negative (purifying) selection with the ω ratio less than 1 (Table 2).

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Gene Flow and Genetic Differentiation among Populations

Kst* will be near zero if there is no genetic differentiation (null hypothesis) (32). A smaller Z* means a smaller genetic differentiation among the population (29). The value of Snn describes a range of the exact same population (value of 0.5) (null hypothesis) to distinctly differentiate the population (value of 1). The Ks *, Kst*, Z*, and *Snn* values obtained from each *CP* gene comparison among their respective populations were all different, which means these populations are distinct from each other. *Fst* value, which is a measure of population differentiation, was calculated for all the geographical populations. *Fst* in all the populations varies from 0.05861 (between American and South Asian populations) to 0.35755 (between East Asia and West Asia populations) (Table 3). Pairwise FST values didn't show a strong genetic differentiation between ASGV populations (Table 3).

In addition gene flow (Nm) in all the populations varied from 4.0 (between American and South Asian populations) to 0.45 (between East Asia and West Asia populations) (Table 3). When the population of West Asia was compared with other populations, the value of *Fst* ranged from 0.10211 to 0.35755 with an Nm value range of 0.45 to 2.02 indicating these populations are differentiated with intermediate to high gene flow. The highest gene flow was determined between America and South Asia (Table 3).

Moderate or low genetic differentiation, and frequent gene flow (FST < 0.33 and Nm > 1) also confirmed with Ks*, Kst* Z, Z* and Snn statistics values (Table 3).

Discussion

The apple and pear are the most economically and culturally significant fruits in the world, and they grow in all temperate zones. Because of its easy transmission, worldwide distribution, long latency, difficult eradication, and usual symptomless, ASGV is considered as one of the main viral pathogens of fruit trees (15).

This virus has been previously reported in West Azerbaijan, Khorasan Razavi (18), Zanjan, and Kurdistan provinces, Iran (19).

However, further work is needed to understand the distribution and prevalence of ASGV from other parts of Iran. In this study, we investigated the presence of ASGV in apple and pear orchards in two counties (Kiyasar and Nowsar) in Mazandaran province, North Iran. ELISA tests showed that the virus is common in the apple and pear trees.

The overall ASGV infection was 10.2%, and it was detected in asymptomatic apple trees in both Kiyasar (17.39%) and Nowsar (7.5%) counties (Table 1). Among pear leaf samples, only symptomatic samples showed the infection with AGV which confirmed by ELISA and RT-PCR.

Table 3. Genetic differentiation measurement between populations from pairwise comparison of ASGV sequences based on geographical isolation

	Ks*	Kst*	*Z**	*Snn*	*P* value for Ks*, Kst*, *Z**, *Snn*	F_{ST}	*Nm*
WA and EA	3.87677	0.01654	9.10649	0.97854	0.1038 ns	0.35755	0.45
WA and SE	3.31243	0.01477	7.40225	0.93673	0.0124 *	0.10211	2.02
WA and AM	3.17260	0.05618	4.72755	0.75298	0.2600 ns	0.19434	1.04
EA and SE	3.78203	0.04160	9.51896	0.98382	0.0145 *	0.18348	1.11
EA and AM	3.91891	0.00630	9.22051	0.95607	0.0983 ns	0.08846	2.58
SE and AM	3.44942	0.01198	7.54782	0.90585	0.0123 *	0.05861	4.0
All	3.88301	0.00670	9.97201	0.93894	1.0000 ns	0.24702	0.76

Probability (p value) obtained by the permutation test (PM test) with 1000 replicates. *, 0.01 < P < 0.05; **, 0.001 < P < 0.01; ***, P < 0.001; ns, not significant.

The PM test was performed using DnaSP v. 6.10.04. FST > 0.33 indicates infrequent gene flow; FST < 0.33 suggests frequent gene flow. Nm is the migration fraction per generation.

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Based on previous studies, ASGV usually does not induce visible symptoms in infected plants, as confirmed by this study, although the infection causes significant yield loss (5, 32).

The negative results for some of the symptommatic samples may be due to a low virus titer, or they could possibly be related to a possible infection with other viruses. (22, 33).

Using the *CP* gene, ASGV isolates are clustered into two main groups (Fig. 2), which is in accordance with the previous report (17). All Iranian ASGV isolates fell into the main GI but in different branches (Fig. 2) as reported by Mohammadlou et al. (19). Molecular analysis of recently reported Chinese isolates using the *CP* gene and full genome sequences indicates three and four phylogroups, respectively (33, 34). Nevertheless, no clustering was found according to the hosts or where the ASGV was isolated, which may refer to the distribution of infected vegetative material (e.g. scions) around the world (33, 35) and the rapid dispersal of ASGV isolates in nature. However, high molacular variability, and different ASGV hosts in East Asian population in comparison with others, may indicates ASGV originated from East Asia. In addition, the role of unknown vector(s) in the distribution and evolution of ASGV cannot be ruled out (36).

The potential recombination sites in CP nt sequences, ASGV isolates were assessed using RDP4. No 'significant' evidence of recombination event(s) (*P* value of $<1\times10^{-6}$) was obtained in the *CP* gene region of Iranian isolates. Recombination may be a relatively frequent process at the molecular level but if most recombinants are deleterious, as is clearly the case for most point mutations, they will be removed by purifying selection and so invisible to any sequence analyses as shown for *Cucumber mosaic virus*-CMV population (37). This may be particularly important in genes like the capsid which play multiple roles in the viral life-cycle and where most recombinants are likely to have severe fitness costs (38). Furthermore, an alternative explanation for the lack of recombination among Iranian isolates may be related to the low number of isolates and/or genomic region has been analyzed.

The role of negative selection pressure in the genetic variation of this virus can be inferred from dN/dS values (Table 2). Generally, low nucleotide diversity, high nucleotide identity (91 to 100%) (Fig. 3), high haplotype diversities, frequent gene flow and low genetic differentiation among populations also indicate the recent distribution of ASGV (Table 3).

Fu's Fs test was not significant for any geographic region ($P > 0.05$), while Tajima's D was significant only in the West Asia population ($P = 0.018$).

The negative values of Tajima's D suggest a possible recent population expansion or from genetic hitchhiking. On the other hand, positive Tajima's D values associated with multimodal mismatch distributions point to a possible population bottleneck and/or population sub-division.

The results of Tajima's D test and Fu's Fs test are presented in Table 2, including associated simulated p-values. Tajima's D test and Fu's FS tests resulted in negative values for all except American population, which was negative but not significant.

Altogether, all ASGV populations appear to be at demographic equilibrium since Tajima's D, Fu's Fs statistics were not statistically significant ($P > 0.10$) in any populations.

Larger sample sizes in future might provide more information to confirm or refute these inference of possible demographic history.

As shown in Table 3, no significant genetic differentiation and frequent gene flow were identified among ASGV populations except between South Asia and East Asia (Table 3). Based on these test statistics, geographical isolation may not have played a role in ASGV population structure, which consistent with the results from the previous studies (17, 19).

Conclusion

The investigation on the distribution of ASGV and its molecular properties can give us more information about the epidemiology and control of this latent and graft-transmissible virus. Furthermore, the prevalence of ASGV shows that virus prevention and control may be poorly managed in orchards. Since it is not possible

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to eliminate virus from infected trees, virus control is reduced to propagation through healthy scions and virus-resistant rootstocks. ASGV-infected trees are visually indistinguishable from healthy trees; therefore timely virus detection and virus source removal are effective management measures.

To our knowledge, this is the first report of ASGV infecting apple and pear trees in North Iran. This finding not only could enrich the knowledge of distribution, biology, and molecular properties of ASGV in Iran but also provided a significant basis for disease management.

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None.

Conflict of Interest

No conflict of interest is declared.

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